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⑲ Applicant: Mycogen Corporation
5451 Oberlin Drive
San Diego, CA 92121(US)

⑳ Inventor: Edwards, David L
13824 Recuerdo Drive
Del Mar California 92014(US)
Inventor: Hermstadt, Corinna
8448 New Salem St., No. 6
San Diego, California 92126(US)
Inventor: Wilcox, Edward R.
2623 Mary Lane Place
Escondido, California 92025(US)
Inventor: Wong, Siu-Yin
7116 Cather Court
San Diego, California 92122(US)

㉑ Representative: Perry, Robert Edward et al
GILL JENNINGS & EVERY 53-64 Chancery
Lane
London WC2A 1HN(GB)

㉒ Bacillus thuringiensis toxins.

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(71) Applicant: Mycogen Corporation
5451 Oberlin Drive
San Diego, CA 92121(US)

(72) Inventor: Edwards, David L.
13824 Recuerdo Drive
Del Mar California 92014(US)
Inventor: Herrnstadt, Corinna
8448 New Salem St., No. 6
San Diego, California 92126(US)
Inventor: Wilcox, Edward R.
2623 Mary Lane Place
Escondido, California 92025(US)
Inventor: Wong, Siu-Yin
7116 Cather Court
San Diego, California 92122(US)

(74) Representative: Perry, Robert Edward et al
GILL JENNINGS & EVERY 53-64 Chancery
Lane
London WC2A 1HN(GB)

(54) **Bacillus thuringiensis toxins.**

(57) A process for altering the insect host range (spectrum) of pesticidal toxins comprises recombining in vitro the variable region(s) (non-homologous) of two or more genes encoding a pesticidal toxin. Specifically exemplified is the recombining of the variable regions of two genes obtained from well-known strains of Bacillus thuringiensis var. kurstaki. The resulting products are chimeric toxins which are shown to have an expanded and/or amplified insect host range as compared to the parent toxins.

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BACILLUS THURINGIENSIS TOXINS

The most widely used microbial pesticides are derived from the bacterium Bacillus thuringiensis. This bacterial agent is used to control a wide range of leaf-eating caterpillars, Japanese beetles and mosquitos. Bacillus thuringiensis produces a proteinaceous paraspore or crystal which is toxic upon ingestion by a susceptible insect host. For example, B. thuringiensis var. kurstaki HD-1 produces a crystal called a delta toxin which is toxic to the larvae of a number of lepidopteran insects. The cloning and expression of the B.t. crystal protein gene in Escherichia coli has been described in the published literature (Schnepf, H.E. and Whiteley, H.R. [1981] Proc. Natl. Acad. Sci. USA 78:2893-2897). U.S. Patent 4,448,885 and U.S. Patent 4,467,036 both disclose the expression of B.t. crystal protein in E. coli. In U.S. 4,467,036 B. thuringiensis var. kurstaki HD-1 is disclosed as being available from the well-known NRRL culture repository at Peoria, Illinois. Its accession number there is NRRL B-3792. B. thuringiensis var. kurstaki HD-73 is also available from NRRL. Its accession number is NRRL B-4488.

Brief Summary of the Invention

The subject invention concerns a novel process for altering the insect host rang of Bacillus thuringiensis toxins, and novel toxins produced as exemplification of this useful process. This alteration can result in expansion of the insect host range of the toxin, and/or, amplification of host toxicity. The process comprises recombining in vitro the variable region(s) of two or more δ -endotoxin genes. Specifically exemplified is the recombining of portions of two Bacillus thuringiensis var. kurstaki DNA sequences, i.e., referred to herein as k-1 and k-73, to produce chimeric B.t. toxins with expanded host ranges as compared to the toxins produced by the parent DNA's.

"Variable regions," as used herein, refers to the non-homologous regions of two or more DNA sequences. As shown by the examples presented herein, the recombining of such variable regions from two different B.t. DNA sequences yields, unexpectedly, a DNA sequence encoding a δ -endotoxin with an expanded insect host range. In a related example, the recombining of two variable regions of two different B.t. toxin genes results in the creation of a chimeric toxin molecule with increased toxicity toward the target insect. The utility of this discovery by the inventors is clearly broader than the examples disclosed herein. From this discovery, it can be expected that a large number of new and useful toxins will be produced. Thus, though the subject process is exemplified by construction of chimeric toxin-producing DNA sequences from two well-known B.t. kurstaki DNA sequences, it should be understood that the process is not limited to these starting DNA sequences. The invention process also can be used to construct chimeric toxins from any B thuringiensis toxin-producing DNA sequence.

Materials and Methods

Upon recombining in vitro the variable region(s) of two or more δ -endotoxin genes, gene(s) is/are obtained, which encode chimeric toxin(s) having expanded and/or amplified host toxicity as compared to the toxin produced by the starting genes. This recombination is done using standard well-known genetic engineering techniques.

The restriction enzymes disclosed herein can be purchased from Bethesda Research Laboratories, Gaithersburg, MS, USA, or New England Biolabs, Beverly, MA, USA. The enzymes are used according to the instructions provided by the supplier.

The various methods employed in the preparation of the plasmids and transformation of host organisms are well-known in the art. These procedures are all described by Maniatis et al (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, USA. Thus, it is within the skill of those in the genetic engineering art to extract DNA from microbial cells, perform restriction enzyme digestions, electrophorese DNA fragments, tail and anneal plasmid and insert DNA, ligate DNA, transform cells, prepare plasmid DNA, electrophorese proteins, and sequence DNA.

Plasmids pEW1, pEW2, pEW3 and pEW4 have been constructed as described below, and have been deposited in E. coli hosts on 29th November 1985 in the permanent collection of the Northern Regional Research Laboratory (NRRL), U.S. Department of Agriculture, Peoria, Illinois, USA.

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5451 Oberlin Drive
San Diego, CA 92121(US)(72) Inventor: Edwards, David L.
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Del Mar California 92014(US)
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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	EP-A-0 063 949 (THE BOARD OF REGENTS OF THE UNIVERSITY OF WASHINGTON) ---		C 12 N 15/00 C 12 P 21/02 A 01 N 63/00 C 12 N 1/20
A	GENE, vol. 36, no. 3, 1985, pages 289-300, Elsevier Science Publishers, Amsterdam, NL; M.J. ADANG et al.: "Characterized full-length and truncated plasmid clones of the crystal protein of <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> HD-73 and their toxicity to <i>Manduca sexta</i> " ---		
P,A	GENE, vol. 43, no. 1/2, 1986, pages 29-40, Elsevier Science Publishers B.V., Amsterdam, NL; J.W. KRONSTAD et al.: "Three classes of homologous <i>Bacillus thuringiensis</i> crystal-protein genes" ---		
P,A	GENE, vol. 48, 1986, pages 109-118, Elsevier Science Publishers B.V. Amsterdam, NL; M. GEISER et al.: "The hypervariable region in the genes coding of entomopathogenic crystal proteins of <i>Bacillus thuringiensis</i> : nucleotide sequence of the <i>kurhd 1</i> gene of subsp. <i>kurstaki</i> HD1" ---		TECHNICAL FIELDS SEARCHED (Int. Cl.4) C 12 N
T	EP-A-0 206 613 (REPLIGEN CORP.) -----		
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 01-02-1988	Examiner PULAZZINI A.F.R.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document			

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The four plasmids are shown schematically in the accompanying Figures 1 to 4. pEW2 and pEW3, having the respective accession number NRRL B-18033 and NRRL B-18034, were deposited on 29th November 1985. pEW1 and pEW4, having the respective accession numbers NRRL B-18134 and NRRL B-18135, were deposited on 13th November 1986. Further, B. thuringiensis strain MTX-36, NRRL B-18101, was deposited on 25th August 1986.

Plasmid pBR322 is a well-known and available plasmid. It is maintained in the E. coli host ATCC 37017. Purified pBR322 DNA can be obtained as described by Bolivar et al (1977) Gene 2:95-133, and by Sutcliffe (1978) Nucleic Acids Res. 5:2721-2728.

As disclosed above, any B. thuringiensis toxin-producing DNA sequence can be used as starting material for the subject invention. Examples of B. thuringiensis organisms, other than those previously given, are as follows:

Bacillus thuringiensis var. israelensis--ATCC 35646

Bacillus thuringiensis M-7--NRRL B-15939

Bacillus thuringiensis var. tenebrionis--DSM 2803

The following B. thuringiensis cultures are available from the United States Department of Agriculture - (USDA) at Brownsville, Texas. Requests should be made to Joe Garcia, USDA, ARS, Cotton Insects Research Unit, P.O. Box 1033, Brownsville, Texas 78520 USA.

B. thuringiensis HD2

B. thuringiensis var. finitimus HD3

B. thuringiensis var. alesti HD4

B. thuringiensis var. kurstaki HD73

B. thuringiensis var. sotto HD770

B. thuringiensis var. dendrolimus HD5

B. thuringiensis var. kenyae HD7

B. thuringiensis var. galleriae HD29

B. thuringiensis var. canadensis HD224

B. thuringiensis var. entomocidus HD9

B. thuringiensis var. subtoxicus HD109

B. thuringiensis var. aizawai HD11

B. thuringiensis var. morrisoni HD12

B. thuringiensis var. ostrinae HD501

B. thuringiensis var. tolworthi HD537

B. thuringiensis var. darmstadiensis HD146

B. thuringiensis var. toumanoffi HD201

B. thuringiensis var. kyushuensis HD541

B. thuringiensis var. thompsoni HD542

B. thuringiensis var. pakistani HD395

B. thuringiensis var. israelensis HD567

B. thuringiensis var. indiana HD521

B. thuringiensis var. dakota

B. thuringiensis var. tohokuensis HD866

B. thuringiensis var. kumanotoensis HD867

B. thuringiensis var. tochiensis HD868

B. thuringiensis var. colmeri HD847

B. thuringiensis var. wuhanensis HD525

Though the main thrust of the subject invention is directed toward a process for altering the host range of B. thuringiensis toxins, the process is also applicable in the same sense to other Bacillus toxin-producing microbes. Examples of such Bacillus organisms which can be used as starting material are as follows:

Bacillus cereus--ATCC 21281

Bacillus moritai--ATCC 21282

Bacillus popilliae--ATCC 14706

Bacillus lentimorbus--ATCC 14707

Bacillus sphaericus--ATCC 33203

Bacillus thuringiensis M-7, exemplified herein, is a Bacillus thuringiensis isolate which, surprisingly, has activity against beetles of the order Coleoptera but not against Trichoplusia ni, Spodoptera exigua or Aedes aegypti. Included in the Coleoptera are various Diabrotica species (family Chrysomelidae) that are responsible for large agricultural losses, for example, D. undecimpunctata (western spotted cucumber beetle), D. longicornis (northern corn rootworm), D. virgata (western corn rootworm), and D. undecimpunctata howardi (southern corn rootworm).

B. thuringiensis M-7 is unusual in having a unique parasporal body (crystal) which under phase contrast microscopy is dark in appearance with a flat, square configuration.

The pesticide encoded by the DNA sequence used as starting material for the invention process can be any toxin produced by a microbe. For example, it can be a polypeptide which has toxic activity toward a eukaryotic multicellular pest, such as insects, e.g., coleoptera, lepidoptera, diptera, hemiptera, dermaptera, and orthoptera; or arachnids; gastropods; or worms, such as nematodes and platyhelminths. Various susceptible insects include beetles, moths, flies, grasshoppers, lice, and earwigs.

Further, it can be a polypeptide produced in active form or a precursor or proform requiring further processing for toxin activity, e.g., the novel crystal toxin of B. thuringiensis var. kurstaki, which requires processing by the pest.

The constructs produced by the process of the invention, containing chimeric toxin-producing DNA sequences, can be transformed into suitable hosts by using standard procedures. Illustrative host cells may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and -positive, include Enterobacteriaceae, such as Escherichia, Erwinia, Shigella, Salmonella, and Proteus; Bacillaceae; Rhizobiaceae, such as Rhizobium; Spirillaceae, such as photobacterium Zymomonas, Serratia, Aeromonas, Vibrio, Desulfovibrio, Spirillum; Lactobacillaceae; Pseudomonadaceae, such as Pseudomonas and Acetobacter; Azotobacteraceae and Nitrobacteriaceae. Among eukaryotes are fungi, such as Phycomycetes and Ascomycetes, which includes yeast, such as Saccharomyces and Schizosaccharomyces; and Basidiomycetes yeast, such as Rhodotorula, Aureobasidium, Sporobolomyces, and the like.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the chimeric toxin-producing gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Host organisms of particular interest include yeast, such as Rhodotorula sp., Aureobasidium sp., Saccharomyces sp., and Sporobolomyces sp.; phylloplane organisms such Pseudomonas sp., Erwinia sp. and Flavobacterium sp.; or such other organisms as Escherichia, Lactobacillus sp., Bacillus sp., and the like. Specific organisms include Pseudomonas aeruginosa, Pseudomonas fluorescens, Saccharomyces cerevisiae, Bacillus thuringiensis, Escherichia coli, Bacillus subtilis, and the like.

The chimeric toxin-producing gene(s) can be introduced into the host in any convenient manner, either providing for extrachromosomal maintenance or integration into the host genome.

Various constructs may be used, which include replication systems from plasmids, viruses, or centromeres in combination with an autonomous replicating segment (ars) for stable maintenance. Where only integration is desired, constructs can be used which may provide for replication, and are either transposons or have transposon-like insertion activity or provide for homology with the genome of the host. DNA sequences can be employed having the chimeric toxin-producing gene between sequences which are homologous with sequences in the genome of the host, either chromosomal or plasmid. Desirably, in the chimeric toxin-producing gene(s) will be present in multiple copies. See for example, U.S. Patent No. 4,399,216. Thus, conjugation, transduction, transfection and transformation may be employed for introduction of the gene.

A large number of vectors are presently available which depend upon eukaryotic and prokaryotic replication systems, such as ColE1, P-1 incompatibility plasmids, e.g., pRK290, yeast 2 μ plasmid, lambda, and the like.

Where an extrachromosomal element is employed, the DNA construct will desirably include a marker which allows for a selection of those host cells containing the construct. The marker is commonly one which provides for biocide resistance, e.g., antibiotic resistance or heavy metal resistance, complementation providing prototrophy to an auxotrophic host, or the like. The replication systems can provide special properties, such as runaway replication, can involve cos cells, or other special feature.

Where the chimeric toxin-producing gene(s) has transcriptional and translational initiation and termination regulatory signals recognized by the host cell, it will frequently be satisfactory to employ those regulatory features in conjunction with the gene. However, in those situations where the chimeric toxin-producing gene is modified, as for example, removing a leader sequence or providing a sequence which codes for the mature form of the pesticide, where the entire gene encodes for a precursor, it will frequently be necessary to manipulate the DNA sequence, so that a transcriptional initiation regulatory sequence may be provided which is different from the natural one.

A wide variety of transcriptional initiation sequences exist for a wide variety of hosts. The sequence can provide for constitutive expression of the pesticide or regulated expression, where the regulation may be inducible by a chemical, e.g., a metabolite, by temperature, or by a regulatable repressor. See for example, U.S. Patent No. 4,374,927. The particular choice of the promoter will depend on a number of factors, the strength of the promoter, the interference of the promoter with the viability of the cells, the effect of regulatory mechanisms endogenous to the cell on the promoter, and the like. A larger number of promoters are available from a variety of sources, including commercial sources.

The cellular host containing the chimeric toxin-producing pesticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the chimeric toxin-producing gene. These cells may then be harvested in accordance with conventional ways and modified in the various manners described above. Alternatively, the cells can be fixed prior to harvesting.

Host cells transformed to contain chimeric toxin-producing DNA sequences can be treated to prolong pesticidal activity when the cells are applied to the environment of a target pest. This treatment can involve the killing of the host cells under protease deactivating or cell wall strengthening conditions, while retaining pesticidal activity.

The cells may be inhibited from proliferation in a variety of ways, so long as the technique does not deleteriously affect the properties of the pesticide, nor diminish the cellular capability in protecting the pesticide. The techniques may involve physical treatment, chemical treatment, changing the physical character of the cell or leaving the physical character of the cell substantially intact, or the like.

Various techniques for inactivating the host cells include heat, usually 50°C to 70°C; freezing; UV irradiation; lyophilization; toxins, e.g., antibiotics; phenols; anilides, e.g., carbanilide and salicylanilide; hydroxyurea; quaternaries; alcohols; antibacterial dyes; EDTA and amidines; non-specific organic and inorganic chemicals, such as halogenating agents, e.g., chlorinating, brominating or iodinating agents; aldehydes, e.g., glutaraldehyde or formaldehyde; toxic gases, such as ozone and ethylene oxide; peroxide; psoralens; desiccating agents; or the like, which may be used individually or in combination. The choice of agent will depend upon the particular pesticide, the nature of the host cell, the nature of the modification of the cellular structure, such as fixing and preserving the cell wall with crosslinking agents, or the like.

The cells generally will have enhanced structural stability which will enhance resistance to environmental degradation in the field. Where the pesticide is in a proform, the method of inactivation should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of inactivation or killing retains at least a substantial portion of the bioavailability or bioactivity of the toxin.

The method of treating the organism can fulfill a number of functions. First, it may enhance structural integrity. Second, it may provide for enhanced proteolytic stability of the toxin, by modifying the toxin so as to reduce its susceptibility to proteolytic degradation and/or by reducing the proteolytic activity of proteases naturally present in the cell. The cells are preferably modified at an intact stage and when there has been a substantial build-up of the toxin protein. These modifications can be achieved in a variety of ways, such as by using chemical reagents having a broad spectrum of chemical reactivity. The intact cells can be combined with a liquid reagent medium containing the chemical reagents, with or without agitation at temperatures in the range of about -10 to 60°C. The reaction time may be determined empirically and will vary widely with the reagents and reaction conditions. Cell concentrations will vary from about 10E2 to 10E10 per ml.

Of particular interest as chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W.H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of the target pest(s).

For halogenation with iodine, temperatures will generally range from about 0 to 50°C, but the reaction can be conveniently carried out at room temperature. Conveniently, the iodination may be performed using triiodide or iodine at 0.5 to 5% in an acidic aqueous medium, particularly an aqueous carboxylic acid solution that may vary from about 0.5-5M. Conveniently, acetic acid may be used, although other carboxylic acids, generally of from about 1 to 4 carbon atoms, may also be employed. The time for the reaction will generally range from less than a minute to about 24 hrs, usually from about 1 to 6 hrs. Any residual iodine may be removed by reaction with a reducing agent, such as dithionite, sodium thiosulfate, or other reducing agent compatible with ultimate usage in the field. In addition, the modified cells may be subjected to further treatment, such as washing to remove all of the reaction medium, isolation in dry form, and formulation with typical stickers, spreaders, and adjuvants generally utilized in agricultural applications, as is well known to those skilled in the art.

Of particular interest are reagents capable of crosslinking the cell wall. A number of reagents are known in the art for this purpose. The treatment should result in enhanced stability of the pesticide. That is, there should be enhanced persistence or residual activity of the pesticide under field conditions. Thus, under conditions where the pesticidal activity of untreated cells diminishes, the activity of treated cells remains for periods of from 1 to 3 times longer.

The cells can be formulated for use in the environment in a variety of ways. They can be employed as wettable powders, granules, or dusts, by mixing with various inert materials, such as inorganic minerals - (phyllosilicates, carbonates, sulfates, or phosphates) or botanical materials (powdered corncobs, rice hulls, or walnut shells). The formulations can include spreader/sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations can be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, and the like. The ingredients can include rheological agents, surfactants, emulsifiers, dispersants, polymers, and the like.

The pesticidal concentration will vary depending upon the nature of the particular formulation, e.g., whether it is a concentrate or to be used undiluted. The pesticide will generally be present at a concentration of at least about 1% by weight, but can be up to 100% by weight. The dry formulations will have from about 1 to 95% by weight of the pesticide, while the liquid formulations will generally be from about 1 to 60% by weight of the solids in the liquid phase. The formulations will generally have from about 1E2 to 1E8 cells/mg.

The formulations can be applied to the environment of the pest(s), e.g., plants, soil or water, by spraying, dusting, sprinkling, or the like. These formulations can be administered at at least 50 g(liquid or dry), e.g. up to 1 kg, per hectare, as required.

Following are examples which illustrate procedures, for practising the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1—Construction of plasmid pEW1

The k-1 gene is the hd-1 gene described by Schnepf et al. (J. Biol. Chem. 260:6264-6272 1985). The k-1 gene was resected from the 5' end with Bal31 up to position 504. To this position was added a Sall linker (5'GTCGACC3'). The 3' end of the gene was cleaved at position 4211 with the enzyme Nde I and blunt ended with the Klenow fragment of DNA polymerase.

The cloning vector pUC8 (Messing, J. and Vieira, J. [1982] Gene 19:269-276) which can be purchased from Pharmacia, Piscataway, NJ, was cleaved with Sall and EcoRI and cloned into plasmid pBR322 which had been cut with the same enzymes. The trp promoter (Genblock, available from Pharmacia) was blunt ended at the 5' end with Klenow and inserted into this hybrid vector by blunt end ligation of the 5' end to the SmaI site of the vector, and by insertion of the 3' end at the Sall site of the vector. The k-1 gene was then inserted using the Sall site at the 5' end any by blunt end ligation of the 3' end to the PvuII site of the vector. A schematic drawing of this construct, called pEW1, is shown in Fig. 1 of the drawings.

Plasmid pEW1 contains the DNA sequence encoding Bacillus thuringiensis toxin k-1.

Example 2--Construction of plasmid pEW2

The k-73 gene is the HD-73 gene described by Adang et al. (Gene 36:289-300 1985). The k-73 gene was cleaved at position 176 with NsiI. The sequence was then cleaved at position 3212 with HindIII and the 3036 base fragment consisting of residues 176-3212 was isolated by agarose gel electrophoresis.

Plasmid pEW1, prepared as described in Example 1, was also cleaved with HindIII (position 3345 in Table 1) and partially digested with NsiI (position 556 in Table 1). The 3036 base fragment from k-73, disclosed above, was inserted into the NsiI to HindIII region of pEW1 replacing the comparable fragment of the k-1 gene, and creating plasmid pEW2. A schematic diagram of pEW2 is shown in Fig. 2 of the drawings.

Plasmid pEW2 contains the DNA sequence encoding Bacillus thuringiensis toxin k-73.

Example 3--Construction of plasmid pEW3

The k-1 gene was cut with SacI at position 1873. The gene was then submitted to partial digestion with HindIII and the 1427 base fragment consisting of residues 1873 to 3345 was isolated by agarose gel electrophoresis. Plasmid pEW2 was cut with SacI and HindIII and the large fragment representing the entire plasmid minus the SacI to HindIII fragment of the k-2 gene was isolated by agarose gel electrophoresis. The 1427 base fragment from the k-1 gene was then ligated into the SacI to HindIII region of pEW2, creating plasmid pEW3. A schematic diagram of pEW3 is shown in Fig. 3 of the drawings.

Plasmid pEW3 contains the DNA sequence encoding Bacillus thuringiensis chimeric toxin k-73/k-1 - (pHY).

The nucleotide sequence encoding the chimeric toxin is shown in Table 1. The deduced amino acid sequence is shown in Table 1A.

Example 4--Construction of plasmid pEW4

The k-1 gene was cut at position 556 with NsiI. The gene was then cut with SacI at position 1873 and the 1317 base fragment from NsiI to SacI was isolated by agarose gel electrophoresis. Plasmid pEW2 was cut with SacI and then submitted to partial digestion with NsiI. The large fragment representing the entire plasmid, minus the NsiI to SacI region of the k-73 gene, was isolated by agarose gel electrophoresis. The 1317 base NsiI to SacI fragment of gene k-1 was then ligated into NsiI to SacI region of pEW2 to create plasmid pEW4. A schematic diagram of pEW4 is shown in Fig. 4 of the drawings.

The nucleotide sequence encoding the chimeric toxin is shown in Table 2. The deduced amino acid sequence is shown in Table 2A.

Plasmid pEW4 contains the DNA sequence encoding Bacillus thuringiensis chimeric toxin k-1/k-73 - (PYH).

Example 5--Insertion of Chimeric Toxin Genes Into Plants

Genes coding for chimeric insecticidal toxins, as disclosed herein, can be inserted into plant cells using the Ti plasmid from Agrobacterium tumefaciens. Plant cells can then be caused to regenerate into plants - (Zambryski, P., Joos, H., Gentello, C., Leemans, J., Van Montague, M. and Schell, J. [1983] EMBO J. 2:2143-2150; Bartok, K., Binns, A., Matzke, A. and Chilton, M-D. [1983] Cell 32:1033-1043). A particularly useful vector in this regard is pEND4K (Klee, H.J., Yanofsky, M.F. and Nester, E.W. [1985] Bio/Technology 3:637-642). This plasmid can replicate both in plant cells and in bacteria and has multiple cloning sites for passenger genes. Toxin genes, for example, can be inserted into the BamHI site of pEND4K, propagated in E. coli, and transformed into appropriate plant cells.

Example 6--Cloning of *B. thuringiensis* genes into baculoviruses

Genes coding for *Bacillus thuringiensis* chimeric toxins, as disclosed herein, can be cloned into baculoviruses such as *Autographa californica* nuclear polyhedrosis virus (AcNPV). Plasmids can be constructed that contain the AcNPV genome cloned into a commercial cloning vector such as pUC8. The AcNPV genome is modified so that the coding region of the polyhedrin gene is removed and a unique cloning site for a passenger gene is placed directly behind the polyhedrin promoter. Examples of such vectors are pGP-B6874, described by Pennock et al. (Pennock, G.D., Shoemaker, C. and Miller, L.K. [1984] Mol. Cell. Biol. 4:399-406), and pAC380, described by Smith et al. (Smith, G.E., Summers, M.D. and Fraser, M.J. [1983] Mol. Cell. Biol. 3:2156-2165). The genes coding for k-1, k-73, k-73/k-1, k-1/k-73, or other *B. t.* genes can be modified with *Bam* HI linkers at appropriate regions both upstream and downstream from the coding regions and inserted into the passenger site of one of the AcNPV vectors.

Example 7--Chimeric Toxin Denoted ACB-1

Enhanced toxicity against all three insects tested was shown by a toxin denoted ACB-1. The toxin ACB-1 (Table 3A) is encoded by plasmid pACB-1 (Table 3). The insecticidal activity encoded by pACB-1, in comparison with pEW3 (Example 3), is as follows:

Clone	LC ₅₀ (O.D. ₅₇₅ /ml)		
	<i>T. ni</i>	<i>H. zea</i>	<i>S. exigua</i>
pEW3	4.3	23.0	12.3
pACB-1	1.2	3.9	1.2

The above test was conducted using the conditions described previously.

The above results show that the ACB-1 toxin has the best composite activity as compared to the other toxins tested herein against all three insects.

Plasmid pACB-1 was constructed between the variable region of MTX-36, a wild *B. thuringiensis* strain, having the deposit accession number NRRL B-18101, and the variable region of HD-71 as follows: MTX-36; N-terminal to *SacI* site. HD-71; *SacI* site to C-terminal.

Total plasmid DNA was prepared from strain MTX-36 by standard procedures. The DNA was submitted to complete digestion by restriction enzymes *SpeI* and *DraI*. The digest was separated according to size by agarose gel electrophoresis and a 1962 bp fragment was purified by electroelution using standard procedures.

Plasmid pEW2 was purified and digested completely with *SpeI* and then submitted to partial digestion with *DraI*. The digest was submitted to agarose gel electrophoresis and a 4,138 bp fragment was purified by electroelution as above.

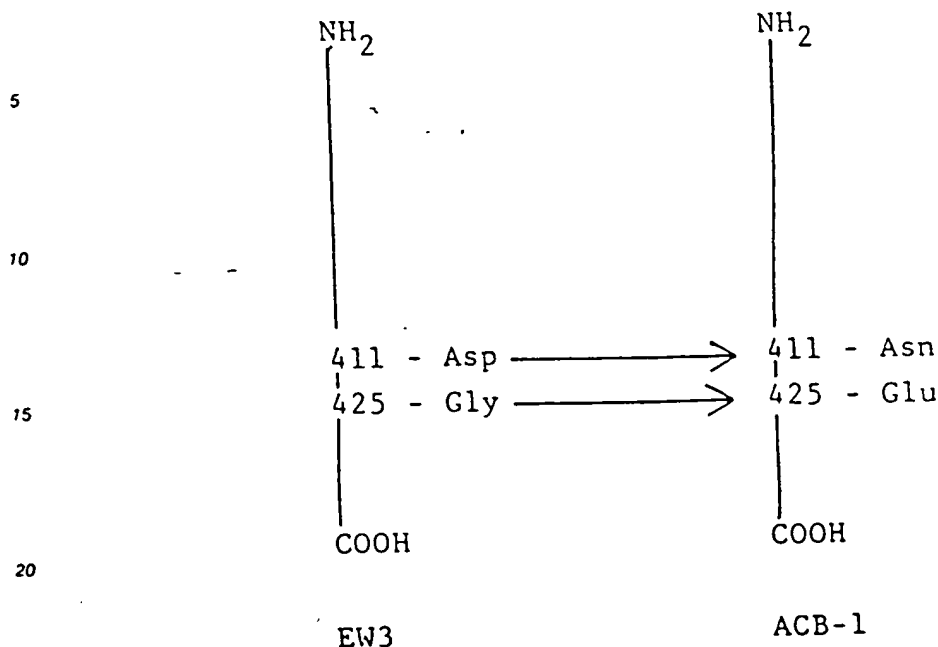
The two fragments (1962 bp from MTX-36 and 4138 bp from pEW2) were ligated together to form construct pACB.

Plasmid DNA was prepared from pACB, digested completely with *SacI* and *NdeI* and a 3760 bp fragment was isolated by electroelution following agarose gel electrophoresis.

Plasmid pEW1 was digested completely with *SacI* and *NdeI* and a 2340 bp fragment was isolated by electroelution following agarose gel electrophoresis.

The two fragments (3760 bp from pACB and 2340 bp from pEW1) were ligated together to form construct pACB-1.

The complete nucleotide sequence of the ACB-1 gene was determined and the deduced amino acid sequence of the toxin was compared with that determined for the toxin encoded by pEW3 (EW3). The result was that the deduced amino acid sequence of the ACB-1 toxin was identical to that of EW3 with two exceptions: (1) Aspartic acid residue 411 in EW3 was changed to asparagine in ACB-1 and (2) glycine residue 425 in EW3 was changed to glutamic acid in ACB-1. These two amino acid changes account for all of the changes in insect toxicity between these strains. The amino acid sequence of the EW3 toxin is as reported in Table 1. A schematic representation of these two toxins is as follows:



25 The above disclosure is further exemplification of the subject invention process for altering the host range of Bacillustoxins which comprises recombining in vitro the variable region of two or more toxin genes. Once a chimeric toxin is produced, the gene encoding the same can be sequenced by standard procedures, as disclosed above. The sequencing data can be used to alter other DNA by known molecular biology procedures to obtain the desired novel toxin. For example, the above-noted changes in the ACB-1 gene from HD-73, makes it possible to construct the ACB-1 gene as follows:

30 Plasmid pEW3, NRRL B-18034, was modified by altering the coding sequence for the toxin. The 151 bp DNA fragment bounded by the AccI restriction site at nucleotide residue 1199 in the coding sequence, and the SacI restriction site at residue 1350 were removed by digestion with the indicated restriction endonucleases using standard procedures. The removed 151 bp DNA fragment was replaced with the

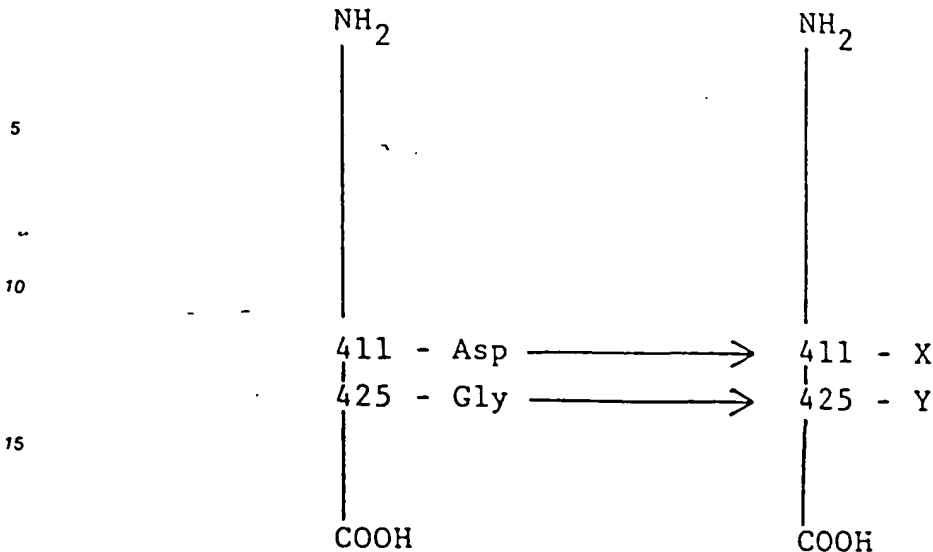
35 following synthetic DNA oligomer by standard procedures:
 A TAC AGA AAA AGC GGA ACG GTA GAT TCG CTG AAT
 GAA ATA CCG CCA CAG AAT AAC AAC GTG CCC CCG
 AGG CAA GAA TTT AGT CAT CGA TTA AGC CAT GTT
 TCA ATG TTT AGA TCT GGC TTT AGT AAT AGT AGT
 40 GTA AGT ATA ATA AGA GCT

The net result of this change is that the aspartic residue at position 411 in the toxin encoded by pEW3 - (Table 1A) is converted to asparagine, and the glycine residue at position 425 is converted to a glutamic residue. All other amino acids encoded by these genes are identical.

45 The changes made at positions 411 and 425, discussed above, clearly illustrate the sensitivity of these two positions in toxin EW3. Accordingly, the scope of the invention is not limited to the particular amino acids depicted as participating in the changes. The scope of the invention includes substitution of all 19 other amino acids at these positions. This can be shown by the following schematic:

50

55



Example 8—Chimeric Toxin Denoted SYW1

Enhanced toxicity against tested insects was shown by a toxin denoted SYW1. The toxin SYW1 (Table 4A) is encoded by plasmid pSYW1 (Table 4). The insecticidal activity encoded by pSYW1, in comparison with pEW1 (Example 1) and pEW2 (Example 2), is as follows:

Clone	LC ₅₀ (O.D. ₅₇₅ /ml)		
	<u>T. ni</u>	<u>H. zea</u>	<u>S. exigua</u>
pEW1	3.5	12.3	18.8
pEW2	1.4	52.3	5.9
pSYW1	0.7	1.9	12.0

The above test was conducted using the conditions described previously.

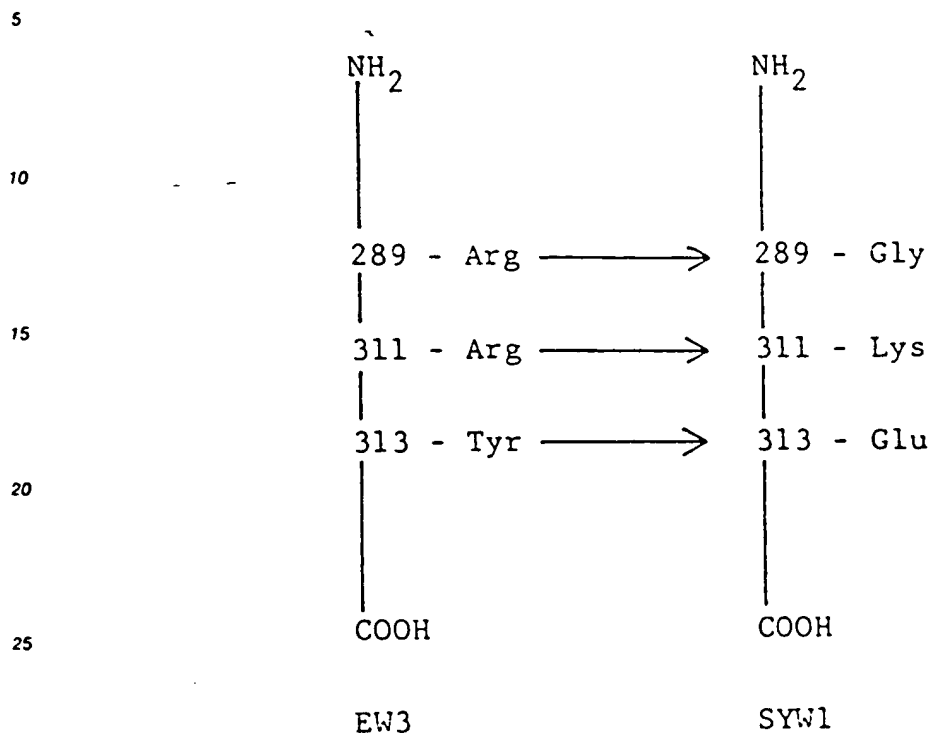
Plasmid pSYW1 was constructed as follows:

Plasmid DNA from pEW2 was prepared by standard procedures and submitted to complete digestion with restriction enzyme AsuI followed by partial digestion with EcoRI. A 5878 bp fragment was purified by electroelution following agarose gel electrophoresis of the digest by standard procedures.

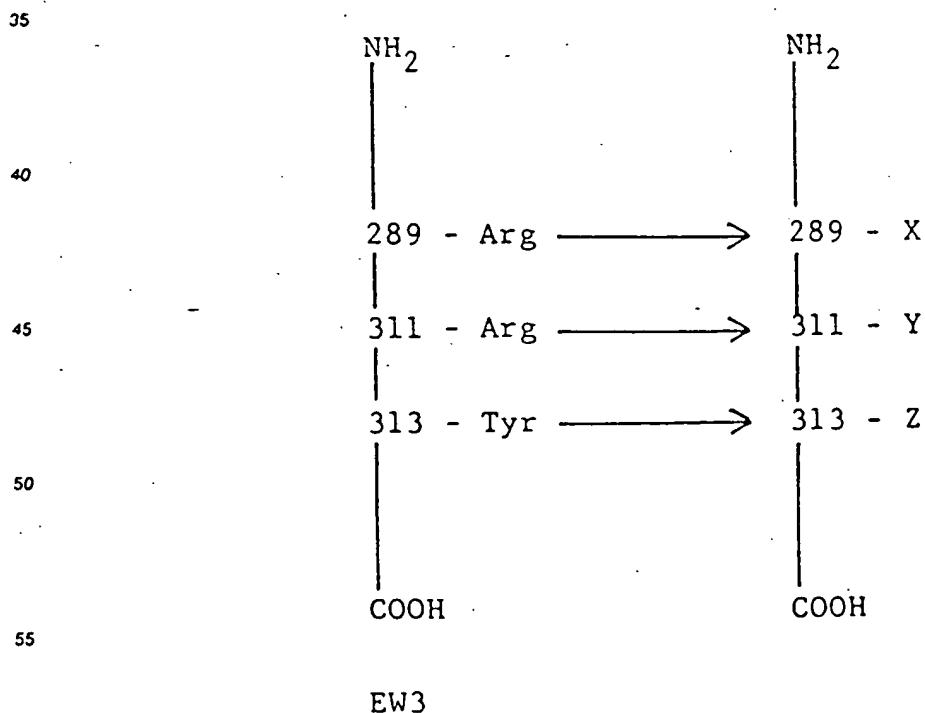
Plasmid DNA from strain HD-1 was prepared and submitted to complete digestion with restriction enzymes AsuI and EcoRI. A 222 bp fragment was purified by electroelution following agarose gel electrophoresis of the digest.

The two fragments (5878 bp from pEW2 and 222 bp from HD-1) were ligated together, by standard procedures, to form construct pSYW1.

The amino acid changes (3) in toxin SYW1 from EW3 are as follows: (1) Arginine residue 289 in EW3 was changed to glycine in SYW1, (2) arginine residue 311 in EW3 was changed to lysine in SYW1, and (3) the tyrosine residue 313 was changed to glycine in SYW1. A schematic representation of these two toxins is as follows:



30 The changes made at positions 289, 311, and 313, discussed above, clearly illustrate the sensitivity of these three positions in toxin EW3. Accordingly, the scope of the invention is not limited to the particular amino acids depicted as participating in the changes. The scope of the invention includes substitution of all the common amino acids at these positions. This can be shown by the following schematic:



wherein X is one of the 20 common amino acids except Arg when the amino acid at position 311 is Arg and the amino acid at position 313 is Tyr; Y is one of the 20 common amino acids except Arg when the amino acid at position 289 is Arg and the amino acid at position 313 is Tyr; and Z is one of the 20 common amino acids except Tyr when the amino acid at position 289 is Arg and the amino acid at position 311 is Arg.

5 Construction of the SYW1 gene can be carried out by procedures disclosed above for the construction of the ACB-1 gene from plasmid pEW3 with appropriate changes in the synthetic DNA oligomer.

As is well known in the art, the amino acid sequence of a protein is determined by the nucleotide sequence of the DNA. Because of the redundancy of the genetic code, i.e., more than one coding nucleotide triplet (codon) can be used for most of the amino acids used to make proteins, different
10 nucleotide sequences can code for a particular amino acid. Thus, the genetic code can be depicted as follows:

Phenylalanine (Phe) TTK

Leucine (Leu) XTY

Isoleucine (Ile) ATM

15 Methionine (Met) ATG

Valine (Val) GTL

Serine (Ser) QRS

Proline (Pro) CCL

Threonine (Thr) ACL

20 Alanine (Ala) GCL

Tyrosine (Tyr) TAK

Termination signal TAJ

Histidine (His) CAK

Glutamine (Gln) CAJ

25 Asparagine (Asn) AAK

Lysine (Lys) AAJ

Aspartic acid (Asp) GAK

Glutamic acid (Glu) GAJ

Cysteine (Cys) TGK

30 Tryptophan (Trp) TGG

Arginine (Arg) WGZ

Glycine (Gly) GGL

Key: Each 3-letter deoxynucleotide triplet corresponds to a trinucleotide of mRNA, having a 5'-end on the left and a 3'-end on the right. All DNA sequences given herein are those of the strand whose sequence
35 corresponds to the mRNA sequence, with thymine substituted for uracil. The letters stand for the purine or pyrimidine bases forming the deoxynucleotide sequence.

A = adenine

G = guanine

C = cytosine

40 T = thymine

X = T or C if Y is A or G

X = C if Y is C or T

Y = A, G, C or T if X is C

Y = A or G if X is T

45 W = C or A if Z is A or G

W = C if Z is C or T

Z = A, G, C or T if W is C

Z = A or G if W is A

QR = TC if S is A, G, C or T; alternatively QR = AG if S is T or C

50 J = A or G

K = T or C

L = A, T, C or G

M = A, C or T

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The above shows that the novel amino acid sequence of the chimeric toxins, and other useful proteins, can be prepared by equivalent nucleotide sequences encoding the same amino acid sequence of the proteins. Accordingly, the subject invention includes such equivalent nucleotide sequences. In addition it has been shown that proteins of identified structure and function may be constructed by changing the amino acid sequence if such changes do not alter the protein secondary structure (Kaiser, E.T. and Kezdy, F.J. [1984] Science 223:249-255). Thus, the subject invention includes muteins of the amino acid sequences depicted herein which do not alter the protein secondary structure.

The one-letter symbol for the amino acids used in Tables 1A and 2A is well known in the art. For convenience, the relationship of the three-letter abbreviation and the one-letter symbol for amino acids is as follows:

Ala	A
Arg	R
Asn	N
Asp	D
Cys	C
Gln	Q
Glu	E
Gly	G
His	H
Ile	I
Leu	L
Lys	K
Met	M
Phe	F
Pro	P
Ser	S
Thr	T
Trp	W
Tyr	Y
Val	V

The work described herein was all done in conformity with physical and biological containment requirements specified in the NIH Guidelines.

CHART A

Bioassay of Chimeric Toxins Against Various Insects

Plasmid	Toxin	LC50 (O.D. 575/ml diet)		
		<u>T. ni</u>	<u>S. exigua</u>	<u>H. zea</u>
pEW1	k-1	3.5	12.3	18.8
pEW2	k-73	1.4	52.3	5.9
pEW3	k-73/k-1	5.7	9.6	10.4
pEW4	k-1/k-73	0.8	30.4	2.2

Recombinant E. coli cells containing the above plasmids were grown overnight in L-broth.* The cells were pelleted and resuspended on 0.85% NaCl. The optical density at 575 nm was determined for these cell suspensions and appropriate dilutions were made in 0.85% NaCl. Three ml of each dilution were added to 27 ml of USDA diet (Dulmage, H.D., Martinez, A.J. and Pena, T [1976] USDA Agricultural Research Service Technical Bulletin No. 1528, U.S. Government Printing Office, Washington, D.C.). The diet/toxin mixture was then dispensed into 24 wells in a plastic tissue culture tray (1.0 ml/well). Single neonate larvae from either Trichoplusia ni, Spodoptera exigua, or Heliothis zea were then added to each well. The trays were then covered with Mylar and punctured with small holes for air exchange. The larvae were observed after 7 days and LC50 values were calculated using the method of probit analysis (Finney, D.J. [1971] Probit Analysis 3rd ed. Cambridge University Press, Cambridge).

* L-broth is 5 g/l NaCl, 10 g/l bactotryptone, 5 g/l yeast extract.

CHART B
Assay of Toxins Against CF-1 Cells in Culture

Plasmid	Toxin	Live Cells (% of Control)	
		Expt. 1	Expt. 2
pEW1	k-1	106%	108%
pEW2	k-73	44%	46%
pEW3	k-73/k-1	105%	97%
pEW4	k-1/k-73	53%	58%

Overnight cultures of E. coli containing the various plasmids were centrifuged and resuspended in 0.85% NaCl containing 1 mM EDTA¹, 0.2 mM PMSF², 0.2 mM TPCK³ and 100 mM NaOH. Cells were broken in a bead beater (Biospec Products, Bartlesville, OK), centrifuged and the supernatant dialyzed against 20 mM Tris-glycine pH 8.5. Toxin was activated with 0.7% trypsin. Assays were carried out on Choristoneura fumiferana cell line CF-1. Approximately 100 µg of activated toxin extract was added to 3.2×10^5 cells in a volume of 1.0 ml. ATP levels were determined after 30 min incubation and the percentage of live cells remaining in the suspension was determined from standard curves.

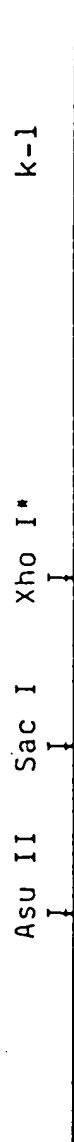
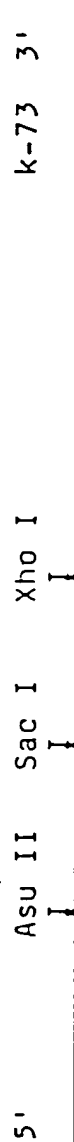
¹ ethylenediaminetetraacetic acid

² phenylmethylsulfonyl fluoride

³ 1-tosylamide-2-phenylethylchloromethyl ketone

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Facile, Comparison of Constructions of Plasmids pEW3 and pEW4



DEW3

7ME4

----- = sequences from k-1
++++ = sequences from k-73
Xho I* means that this restriction site found in k-73 no longer exists in k-1 and will have to be recreated by site specific mutagenesis (it involves changing two base pairs in k-1).

Table 1
Nucleotide Sequence of Plasmid pEW3 Encoding
Chimeric Toxin

Numbering of the nucleotide bases is the same as
Schnepf et al. (J. Biol. Chem. 260:6264-6272 [1985])
for HD-1 and Adang et al. (Gene 36:289-300 [1985])
for HD-73. Only protein coding sequences are shown.

		(start HD-73)	ATG GATAACAATC	400
	CGAACATCAA	TGAATGCATT	CCTTATAATT	GTTTAAGTAA
	GAAGTATTAG	GTGGAGAAAG	AATAGAAACT	GGTTACACCC
20	TTCCTTGTCG	CTAACGCAAT	TTCTTTTGAG	TGAATTTGTT
	GATTTGTGTT	AGGACTAGTT	GATATAATAT	GGGGAATTTT
	CAATGGGACG	CATTTCTTGT	ACAAATTGAA	CAGTTAATTA
	AGAAGAATTC	GCTAGGAACC	AAGCCATTTT	TAGATTAGAA
	ATCTTTATCA	AATTTACGCA	GAATCTTTTA	GAGASTGGGA
25	ACTAATCCAG	CATTAAGAGA	AGAGATGCGT	ATTCAATTCA
	CAGTSCCCTT	ACAACCGCTA	TTCTCTTTT	TGCAGTTCAA
	TTCTCTTTT	ATCAGTATAT	GTTCAAGCTG	CAAATTTACA
	TTGAGAGATG	TTTCAGTGT	TGGACAAAGG	TGGGGATTTG
	TATCAATAGT	CGTTATAATG	ATTTAACTAG	GCTTATTGGC
30	ATTATGCTGT	ACGCTGGTAC	AATACGGGAT	TAGAAGTGT
	GATTCTAGAG	ATTGGGTAA	GTATAATCAA	TTTAGAAGAG
	AACTGTATTA	GATATCGTTG	CTCTGTTCCC	GAATTATGAT
	ATCCAATTCC	AACAGTTTCC	CAATTAACAA	GAGAAATTTA
	GTATTAGAAA	ATTTTGATGG	TAGTTTTGGA	GGCTCGGCTC
35	AAGAAATATT	AGGAGTCCAC	ATTTGATGGA	TATACTTAAC
	TCTATACGGA	TGCTCATAGG	GGTTATTATT	ATTGGTCAGG
	ATGSCCTTCT	CTGTAGGGTT	TTGGGGCCCA	GAATTCACCT
	TGGAACATAT	GGAAATGCAG	CTCCACAACA	ACGTATTGTT
	GTGAGGSCGT	GTATAGAACA	TTATCGTCCA	CTTTATATAG
40	AATATAGGGA	TAAATAATCA	ACAACATCT	GTTCTTGACG
	TGCTTATGGA	ACCTCCTCAA	ATTTGCCATC	CGCTGTATAC
	GAACGGTAGA	TTCGCTGGAT	GAAATACCGC	CACAGAATAA
	CCTAGGCAAG	GATTTAGTCA	TCGATTAAGC	CATGTTTCAA
	AGGCTTTAGT	AATAGTAGTG	TAAGTATAAT	AAGAGCT (end hd-73)
45	(start HD-1)	CCAACGT	TTTCTTGCCA	GCATCGCAGT
	GCTGAATTTA	ATAATATAAT	TCCTTCATCA	CAAATTACAC
	AACAAAATCT	ACTAATCTTG	GCTCTGGAAC	TTCTGTCGTT
	GATTTACAGG	AGGAGATATT	CTTCGAAGAA	CTTCACCTGG
	ACCTTAAGAG	TAAATATTAC	TGCACCATTA	TCACAAAGAT
50	AATTCGCTAC	GCTTCTACTA	CAAATTTACA	ATTCCATACA
	GAAGACCTAT	TAATCAGGGT	AATTTTTCAG	CAACTATGAG
	AATTTACAGT	CCGGAAGCTT	TAGGACTGTA	GGTTTTACTA
	CTTTTCAAAT	GGATCAAGTG	TATTTACGTT	AAGTGCTCAT
	CAGGCAATGA	AGTTTATATA	GATCGAATTG	AATTTGTTCC
55	ACCTTTGAGG	CAGAATATGA	TTTAGAAAGA	GCACAAAAGG
	GCTGTTTACT	TCTTCCAATC	AAATCGGGT	AAAAACAGAT

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Table 1 (cont.)

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ATCATATTGA	TCAAGTATCC	AATTTAGTTG	AGTGTTTATC	AGATGAATTT	2500
TGTCTGGATG	AAAAACAAGA	ATTGTCCGAG	AAAGTCAAAC	ATGCGAAGCG	
ACTTAGTGAT	GAGCGGAATT	TACTTCAAGA	TCCAACTTC	AGAGGGATCA	2600
ATAGACAAC	AGACCGTGGC	TGGAGAGGAA	GTACGGATAT	TACCATCCAA	
GGAGGCGATG	ACGTATTCAA	AGAGAATTAC	GTTACGCTAT	TGGGTACCTT	2700
TGATGAGTGC	TATCCAACGT	ATTTATATCA	AAAAATAGAT	GAGTCGAAAT	
TAAAAGCCTA	TACCCGTTAT	CAATTAAGAG	GGTATATCGA	AGATAGTCAA	2800
GACTTAGAAA	TCTATTTAAT	TCGCTACAAT	GCAAAACATG	AAACAGTAAA	
TGTGCCAGGT	ACGGGTTCCT	TATGGCCGCT	TTCAGCCCAA	AGTCCAATCG	2900
GAAAGTGTGG	AGAGCCGAAT	CGATGCGCGC	CACACCTTGA	ATGGAATCCT	
GACTTAGATT	GTTCTGTAG	GGATGGAGAA	AAGTGTGCC	ATCATTCCGA	3000
TCATTTCTCC	TTAGACATTG	ATGTAGGATG	TACAGACTTA	AATGAGGACC	
TAGGTGTATG	GGTGATCTTT	AAGATTAAGA	CGCAAGATGG	GCACGCAAGA	3100
CTAGGGAATC	TAGAGTTTCT	CGAAGAGAAA	CCATTAGTAG	GAGAAAGCCT	
AGCTCGTGTG	AAAAGAGCGG	AGAAAAATG	GAGAGACAAA	CCTGAAAAAT	3200
TGGAATGGGA	AACAAATATC	GTTTATAAAG	AGGCAAAAGA	ATCTGTAGAT	
GCTTTATTTG	TAAACTCTCA	ATATGATCAA	TTACAAGCGG	ATACGAATAT	3300
TGCCATGATT	CATGCGGCAG	ATAAACGTGT	TCATAGCATT	CGAGAAAGCTT	
ATCTGCCTGA	GCTGTCTGTG	ATTCCGGGTG	TCAATGCGGC	TATTTTGTAA	3400
GAATTAGAAG	GGCGTATTTT	CACTGCATTG	TCCCTATATG	ATGCGAGAAA	
TGTCATTAAA	AATGGTGATT	TTAATAATGG	CTTATCCTGC	TGGAACGTGA	3500
AAGGGCATGT	AGATGTAGAA	GAACAAAACA	ACCAACGTTT	GGTCCTTGTT	
CTTCCGGAAT	GGGAAGCAGA	AGTGTCACAA	GAAGTTCGTG	TCTGTCCGGG	3600
TCGTGGCTAT	ATCCTTCGTG	TCACAGCGTA	CAAGGAGGGA	TATGGAGAAG	
GTTGCGTAAC	CATTATGAG	ATCGAGAACA	ATACAGACGA	ACTGAAGTTT	3700
AGCAACTGCG	TAGAAGAGGA	AATCTATCCA	AATAACACGG	TAACGTGTAA	
TGATTATACT	GTAATCAAG	AAGAATACGG	AGGTGCGTAC	ACTTCTCGTA	3800
ATCGAGGATA	TAACGAAGCT	CCTTCCGTAC	CAGCTGATTA	TGCGTCAGTC	
TATGAAGAAA	AATCGTATAC	AGATGGACGA	AGAGAGAATC	CTTGTGAATT	3900
TAACAGAGGG	TATAGGGATT	ACACGCCACT	ACCAATTGGT	TATGTGACAA	
AAGAATTAGA	ATACTTCCCA	GAAACCGATA	AGGTATGGAT	TGAGATTGGA	4000
GAAACGGAAG	GAACATTTAT	CGTGGACAGC	GTGGAATTAC	TCCTTATGGA	

GGAA (end HD-1)

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Table 1A
Deduced Amino Acid Sequence of Chimeric Toxin Produced
by Plasmid pEW3

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M D M N F N I N E C I P Y N C L S N F E V E V L G G E R I E
T G Y T F I D I S L S L T Q F L L S E F V F G A G F V L G L
V D I I W G I F G F S Q W D A F L V D I E Q L I N O R I E E
F A R N D A I S R L E G L S N L Y Q I Y A E S F R E W E A D
P T N P A L R E E M R I Q F N D M N S A L T T A I F L F A V
Q N Y Q V F L L S V Y V Q A A N L H L S V L R D V S V F G Q
R W G F D A A T I N S R Y N D L T R L I G N Y T D Y A V R W
Y N T G L E R V W G F D S R D W V R Y N Q F R R E L T L T V
L D I V A L F F N Y D S R R Y P I R T V S Q L T R E I Y T N
P V L E N F D G S F R G S A Q G I E R S I R S P H L M D I L
N S I T I Y T D A H R G Y Y Y W S G H Q I M A S P V G F S G
P E F T F P L Y G T M G N A A P Q Q R I V A Q L G Q G V Y R
T L S S T L Y R R F F N I G I N N Q Q L S V L D G T E F A Y
G T S S N L P S A V Y R K S G T V D S L D E I P P O N N N V
P P R Q G F S H R L S H V S M F R S G F S N S S V S I I R A
P T F S W Q H R S A E F N N I I P S S Q I T Q I P L T K S T
N L G S G T S V V K G P G F T G G D I L R R T S P G Q I S T
L R V N I T A P L S Q R Y R V R I R Y A S T T N L Q F H T S
I D G R F I N Q G N F S A T M S S G S N L Q S G S F R T V G
F T T F F N F S N G S S V F T L S A H V F N S G N E V Y I D
R I E F V P A E V T F E A E Y D L E R A Q K A V N E L F T S
S N Q I G L K T D V T D Y H I D Q V S N L V E C L S D E F C
L D E K Q E L S E K V K H A K R L S D E R N L L Q D F N F R
G I N R Q L D R G W R G S T D I T I D G G D D V F K E N Y V
T L L G T F D E C Y P T Y L Y Q K I D E S K L K A Y T R Y Q
L R G Y I E D S Q Q L E I Y L I R Y N A K H E T V N V P G T
G S L W P L S A Q S F I G K C G E P N R C A P H L E W N P D
L D C S C R D G E K C A H H S H H F S L D I D V G C T D L N
E D L G V W V I F K I K T Q D G H A R L G N L E F L E E K P
L V G E A L A R V K R A E K K W R D K R E K L E W E T N I V
Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M I H
A A D K R V H S I R E A Y L P E L S V I P G V N A A I F E E
L E G R I F T A F S L Y D A R N V I K N G D F N N G L S C W
N V K G H V D V E E Q N N Q R S V L V L P E W E A E V S Q E
V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H E I
E N N T D E L K F S N C V E E E I Y P N N T V T C N D Y T V
N Q E E Y G G A Y T S R N R G Y N E A P S V P A D Y A S V Y
E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V G Y
V T K E L E Y F P E T D K V W I E I G E T E G T F I V D S V
E L L L M E E

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Table 2
Nucleotide Sequence of Plasmid pEW4 Encoding
Chimeric Toxin

Numbering of nucleotide bases is the same as Schnepf et al. (J. Biol. Chem. 260:6264-6272 [1985]) for HD-1 and Adang et al. (Gene 36:289-300 [1985]) for HD-73. Only protein coding sequences are shown.

	(start HD-1)	ATGG	ATAACAATCC	GAACATCAAT	
20	GAATGCATTC	CTTATAATTG	TTTAAGTAAC	CCTGAAGTAG	AAGTATTAGG 600
	TEGAGAAAGA	ATAGAAACTG	GTTACACCCC	AATCGATATT	TCCTTGTCTC
	TAACGCAATT	TCTTTTGAGT	GAATTTGTTT	CCGGTGCTGG	ATTTGTGTTA 700
	GGAAGAGTTG	ATATAATATG	GGGAATTTT	GGTCCCTCTC	AATGGGACGC
	ATTTCTGTGA	CAAATTGAAC	AGTTAATTAA	CCAAAGAATA	GAAGAATTCTG 800
	CTAGGAACCA	AGCCATTTCT	AGATTAGAAG	GACTAAGCAA	TCTTTATCAA
25	ATTTACGCAG	AATCTTTTAG	AGAGTGGGAA	GCAGATCCTA	CTAATCCAGC 900
	ATTAAGAGAA	GAGATGCGTA	TTCAATTCAA	TCACATGAAC	AGTGGCCCTTA
	CAACCGCTAT	TCCTCTTTTG	GCAGTTCAAA	ATTATCAAGT	TCCTCTTTTA 1000
	TCAGTATATG	TTCAAGCTGC	AAATTTACAT	TTATCAGTTT	TGAGAGATGT
	TTCAGTGTTC	GGACAAAGGT	GGGGATTTGA	TGCCGCGACT	ATCAATAGTC 1100
30	GTTATAATGA	TTTAAGTAGG	CTTATTGGCA	ACTATACAGA	TTATGCTGTG
	CGCTGGTACA	ATACGGGATT	AGAGCGTGTG	TGGGGACCGG	ATTCTAGAGA 1200
	TTGGGTAAGG	TATAATCAAT	TTAGAAGAGA	GCTAACACTT	ACTGTATTAG
	ATATCGTTGC	TCTATTCTCA	AATTATGATA	GTCGAAGGTA	TCCAATTCEA 1300
	ACAGTTTCCC	AATTAACAAG	AGAAATTTAT	ACGAACCCAG	TATTAGAAAA
35	TTTTGATGGT	AGTTTTCTGT	GAATGGCTCA	GAGAATAGAA	CAGAATATTA 1400
	GGCAACCACA	TCTTATGGAT	ATCCTTAATA	GTATAACCAT	TTATACTGAT
	GTGCATAGAG	GCTTTAATTA	TTGGTCAGGG	CATCAAATAA	CAGCTTCTCC 1500
	TGTAGGGTTT	TCAGGACCAG	AATTCGCATT	CCCTTTATTT	GGGAATGCGG
	GGAAATGCAGC	TCCACCCGTA	CTTGTCTCAT	TAACTGTTTT	GGGGATTTTT 1600
40	AGAACATTAT	CTTCACCTTT	ATATAGAAGA	ATTATACTTG	GTTCAGGCCC
	AAATAATCAG	GAAGTGTGTT	TCCTTGATGG	AACGGAGTTT	TCTTTTGCCT 1700
	CCCTAACGAC	CAACTTGCCT	TCCACTATAT	ATAGACAAAG	GGGTACAGTC
	GATTCACTAG	ATGTAATACC	GCCACAGGAT	AATAGTGTAC	CACCTCGTGC 1800
	GGGATTTAGC	CATCGATTGA	GTCATGTTAC	AATGCTGAGC	CAAGCAECTG
45	GAGCAGTTTA	CACCTTGAGA	GCTCAACGT	(stop HD-1)	
	(start HD-73)		CCT	ATGTTCTCTT	
	GGATACATCG	TAGTGCTGAA	TTTAATAATA	TAATTGCATC	GGATAGTATT 1800
	ACTCAAATCC	CTGCAGTGAA	GGGAAACTTT	CTTTTAAATG	GTTCTGTAAT
	TTCAGGACCA	GGATTTACTG	GTGGGGACTT	AGTTAGATTA	AATAGTAGTG 1900
	GAAATAACAT	TCAGAATAGA	GGGTATATTG	AAGTTCCAAT	TCACTTCCCA
50	TCGACATCTA	CCAGATATCG	AGTTCGTGTA	CGGTATGCTT	CTGTAAACCC 2000
	GATTCACCTC	AACGTTAATT	GGGGTAATTC	ATCCATTTTT	TCCAATACAG
	TACCAGCTAC	AGCTACGTCA	TTAGATAATC	TACAATCAAG	TGATTTTGGT 2100
	TATTTTGAAA	GTGCCAATGC	TTTTACATCT	TCATTAGGTA	ATATAGTAGG
	TGTTAGAAAT	TTTAGTGSGA	CTGCAGGAGT	GATAATAGAC	AGATTTGAAT 2200
55	TTATTCCAGT	TACTGCAACA	CTCGAGGCTG	AATATAATCT	GGAAAGAGCG

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Table 2 (cont.)

15 CAGAAAGCGG TGAATGCGCT GTTTACGTCT ACAAACCAAC TAGGGCTAAA 2300
 AACAAATGTA ACGGATTATC ATATTGATCA AGTGTCCAAT TTAGTTACGT
 ATTTATCGGA TGAATTTTGT CTGGATGAAA AGCGAGAAAT GTCCGAGAAA 2400
 GTCAACCATG CGAAGCGACT CAGTGATGAA CGCAATTTAC TCCAAGATTC
 AAATTTCAAA GACATTAATA GGCAACCAGA ACGTGGGTGG GSCGGAAGTA 2500
 CAGGGATTAC CATCCAAGGA GGGGATGACG TATTTAAAGA AAATTACGTC
 20 ACACATCAG GTACCTTTGA TGAGTGCTAT CCAACATATT TGATCAAAA 2600
 AATCGATGAA TCAAAATTAA AAGCCTTTAC CCGTTATCAA TTAAGAGGGT
 ATATCGAAGA TAGTCAAGAC TTAGAAATCT ATTTAATTGG CTACAATGCA 2700
 AAACATGAAA CAGTAAATGT GCCAGGTACG GGTTCCTTAT GCGCGCTTTC
 AGCCCAAAGT CCAATCGGAA AGTGTGGAGA GCCGAATCGA TGCSCGCCAC 2800
 25 ACCTTGAATG GAATCCTGAC TTAGATTGTT CGTGTAGGGA TGGAGAAAAG
 TGTGCCCATC ATTCGCATCA TTTCTCCTTA GACATTGATG TAGGATGTAC 2900
 AGACTTAAAT GAGGACCTAG GTGTATGGGT GATCTTTAAG ATTAAGACGC
 AAGATGGGCA CGCAAGACTA GGGGAATCTAG AGTTTCTCGA AGAGAAACCA 3000
 TTAGTAGGAG AAGCGCTAGC TCGTGTGAAA AGAGCGGAGA AAAAATGGAG
 30 AGACAAACGT GAAAAATTGG AATGGGAAAC AAATATCGTT TATAAGAGGG 3100
 CAAAAGAATC TGATAGTGCT TTATTTGTAA ACTCTCAATA TGATCAATTA
 CAAGCGGATA CGAATATTGC CATGATTCAT GCGGCAGATA AACGTGTTCA 3200
 TAGCATTGCA GAAGCTTATC TGCTGAGCT GTCTGTGATT CCGGGTGTCA
 ATGCGGCTAT TTTTGAAGAA TTAGAAGGGC GTATTTTCAC TGCATTCTCC 3300
 35 CTATATGATG CGAGAAATGT CATTAAAAAT GGTGATTTTA ATAATGGCTT
 ATCCTGCTGG AACGTGAAAG GGCATGTAGA TGTAGAAGAA CAAAACAACC 3400
 AACGTTGGGT CTTGTTGTT CCGGAATGGG AAGCAGAAGT GTCACAAGAA
 GTTCGTGTCT GTCCGGGTGG TGGCTATATC CTTGCTGTCA CAGCGTACAA 3500
 GGAGGGATAT GGAGAAGGTT GCGTAACCAT TCATGAGATC GAGAACATA
 40 CAGACGAATC GAAGTTTATC AACTGCSTAG AAGAGGAAAT CTATCCAAAT 3600
 AACACGGTAA CGTGTAAATG TTATACTGTA AATCAAGAAG AATACGGAGG
 TGGGTACACT TCTCGTAATC GAGGATATAA CGAAGCTCCT TCCGTACCAG 3700
 CTGATTATGC GTCAGTCTAT GAAGAAAAAT CGTATACAGA TGGACGAAGA
 GAGAATCCTT GTGAATTTAA CAGAGGGTAT AGGGATTACA CGCCACTACC 3800
 45 AGTTGGTTAT GTGACAAAAG AATTAGAATA CTTCCAGAA ACCGATAAGG
 TATGGATTGA GATTGGAGAA ACGGAAGGAA CATTTATCGT GACAGCGCTG 3900
 GAATTACTCC TTATGGAGGA A (end HD-73)

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Table 2A

10 Deduced Amino Acid Sequence of Chimeric Toxin Produced
by Plasmid pEW4

15 M D M N P N I N E C I F Y N C L S N P E V E V L G G E R I E
T G Y T P I D I S L S L T Q F L L S E F V P G A G F V L G L
V D I I W G I F G P S Q W D A F P V Q I E Q L I N Q R I E E
F A R N Q A I S R L E G L S N L Y Q I Y A E S F R E W E A D
P T N P A L R E E M R I Q F N D M N S A L T T A I P L L A V
Q N Y Q V P L L S V Y V Q A A N L H L S V L R D V S V F G Q
20 R W G F D A A T I N S R Y N D L T R L I G N Y T D Y A V R W
Y N T G L E R V W G P D S R D W V R Y N Q F R R E L T L T V
L D I V A L F S N Y D S R R Y P I R T V S Q L T R E I Y T N
P V L E N F D G S F R G M A Q R I E Q N I R Q P H L M D I L
N S I T I Y T D V H R G F N Y W S G H Q I T A S P V G F S G
25 P E F A F P L F G N A G N A A P P V L V S L T G L G I F R T
L S S P L Y R R I I L G S G P N N Q E L F V L D G T E F S F
A S L T T N L P S T I Y R Q R G T V D S L D V I P P Q D N S
V P F R A G F S H R L S H V T M L S Q A A G A V Y T L R A Q
R P M F S W I H R S A E F N N I I A S D S I T Q I P A V K G
30 N F L F N G S V I S G P G F T G G D L V R L N S S G N N I Q
N R G Y I E V P I H F F S T S T R Y R V R V R Y A S V T P I
H L N V N W G N S S I F S N T V P A T A T S L D N L Q S S D
F G Y F E S A N A F T S S L G N I V G V R N F S G T A G V I
I D R F E F I P V T A T L E A E Y N L E R A Q K A V N A L F
35 T S T N Q L G L K T N V T D Y H I D Q V S N L V T Y L S D E
F C L D E K R E L S E K V K H A K R L S D E R N L L Q D S N
F K D I N R Q P E R G W G G S T G I T I Q G G D D V F K E N
Y V T L S G T F D E C Y P T Y L Y Q K I D E S K L K A F T R
Y Q L R G Y I E D S Q D L E I Y L I R Y N A K H E T V N V P
40 G T G S L W P L S A Q S P I G K C G E F N R C A P H L E W N
P D L D C S C R D G E K C A H H S H H F S L D I D V G C T D
L N E D L G V W V I F K I K T Q D G H A R L G N L E F L E E
K P L V G E A L A R V K R A E K K W R D K R E K L E W E T N
I V Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M
45 I H A A D K R V H S I R E A Y L P E L S V I P G V N A A I F
E E L E G R I F T A F S L Y D A R N V I K N G D F N N G L S
C W N V K G H V D V E E Q N N Q R S V L V V P E W E A E V S
Q E V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H
E I E N N T D E L K F S N C V E E E I Y F N N T V T C N D Y
50 T V N Q E E Y G G A Y T S R N R G Y N E A P S V P A D Y A S
V Y E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V
G Y V T K E L E Y F P E T D K V W I E I G E T E G T F I V D
S V E L L L M E E

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Table 3

Nucleotide Sequence of Plasmid pACB-1 Encoding
Chimeric Toxin ACB-1

The nucleotide differences as compared to the sequence shown in Table 1 are underlined at positions 1618 and 1661 and code for amino acid changes at positions 411 and 425 as shown in Table 3A.

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15
      (start HD-73)                                ATG GATAACAATC 400
CGAACATCAA TGAATGCATT CCTTATAATT GTTTAAGTAA CCCTGAAGTA
GAASTATTAG GTGGAGAAAG AATAGAAACT GGTACACCCC CAATCSATAT 500
TTCCTTGTCG CTAACGCAAT TTCTTTTGAG TGAATTTGTT CCCGGTGCTG
20 GATTTGTGTT AGGACTAGTT GATATAATAT GGGGAATTTT TGGTCCCTCT 600
CAATGGGACG CATTTCCTGT ACAAAATTGAA CAGTTAATTA ACCAAAGAAAT
AGAAGAATTC GCTAGGAACC AAGCCATTTT TAGATTAGAA GGAATAAGCA 700
ATCTTTATCA AATTTACGCA GAATCTTTTA GAGAGTGGGA AGCAGATCCT
ACTAATCCAG CATTAGAGAG AGAGATGCGT ATTCAATTCA ATGACATGAA 800
25 CAGTGGCCTT ACAACCGCTA TTCCTCTTTT TGCAGTTCAA AATTATCAAG
TTCCTCTTTT ATCAGTATAT GTTCAAGCTG CAAATTTACA TTTATCAGTT 900
TTGAGAGATG TTTCAGTGTT TGGACAAAGG TGGGGATTTG ATGCCGCGAC
TATCAATAGT CGTTATAATG ATTTAACTAG GCTTATTGGC AACTATACAG 1000
ATTATGCTGT ACGCTGGTAC AATACGGGAT TAGAACGTGT ATGGGGACCG
30 GATTCTAGAG ATTGGGTAAG GTATAATCAA TTTAGAAGAG AATTAACACT 1100
AACTGTATTA GATATCGTTG CTCTGTTCCC GAATTATGAT AGTAGAAGAT
ATCCAATTGG AACAGTTTCC CAATTAACAA GAGAAATTTA TACAAACCCA 1200
GTATTAGAAA ATTTTGATGG TAGTTTTCSA GGCTCGGCTC AGGCGATAGA
AAGAAATATT AGGAGTCCAC ATTTGATGGA TATACTTAA AGTATAACCA 1300
35 TCTATACGGA TGCTCATAGG GGTTATTATT ATTGGTCAGG GCATCAATA
ATGGCTTCTC CTGTAGGGTT TTCGGGGCCA GAATTCACCT TTCCGCTATA 1400
TGGAACTATG GGAAATGCAG CTCCACAACA ACGTATTGTT GCTCAACTAG
GTCAGGGCGT GTATAGAACA TTATCGTCCA CTTTATATAG AAGACCTTTT 1500
AATATAGGGA TAAATAATCA ACAACTATCT GTTCTTGACG GGACAGAATT
TGCTTATGGA ACCTCCTCAA ATTTGCCATC CGCTGTATAC AGAAAAAGCG 1600
40 GAACGCTAGA TTCGCTGAAT GAAATACCGC CACAGAATAA CAACGTGCCA
CCTAGGCAAG AATTTAGTCA TCGATTAAGC CATGTTTCAA TGTTTCGTTT 1700
AGGCTTTAGT AATAGTAGTG TAAGTATAAT AAGAGCT (end hd-73)
      (start HD-1)                                CCAACGT TTTCTTGGCA GCATCGCAST 1900
GCTGAATTTA ATAATATAAT TCCTTCATCA CAAATTACAC AAATACSTTT
45 AACAAAATCT ACTAATCTTG GCTCTGGAAC TTCTGTGCTT AAAGGACCAG 2000
GATTTACAGG AGGAGATATT CTTGGAAGAA CTTACCTGGG CCAGATTTC A
ACCTTAAGAG TAAATATTAC TGCACCATTA TCACAAAGAT ATCGGTAAG 2100
AATTCGCTAC GCTTCTACTA CAAATTTACA ATTCCATACA TCAATTGACG
GAAGACCTAT TAATCAGGGT AATTTTTCAG CAACTATGAG TAGTGGGAGT 2200
50 AATTTACAGT CCGGAAGCTT TAGGACTGTA GGTTTTACTA CTCCGTTTAA
CTTTTCAAT GGATCAAGTG TATTTACGTT AAGTGCTCAT GTCTTCAAT 2300
CAGGCAATGA AGTTTATATA GATCGAATTG AATTTGTTCC GGCAGAAGTA
ACCTTTGAGG CAGAATATGA TTTAGAAAGA GCACAAAAGG CGGTGAATGA 2400
GCTGTTTACT TCTTCCAATC AAATCGGGTT AAAACAGAT GTGACGGATT
55 ATCATATTGA TCAAGTATCC AATTTAGTTG AGTGTTTATC AGATGAATTT 2500
TGTCTGGATG AAAACAAGA ATTGTCCGAG AAAGTCAAAC ATGCGAAGCG

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Table 3 (cont.)

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ACTTAGTGAT GAGCGGAATT TACTTCAAGA TCCAAACTTC AGAGGGATCA 2600
 ATAGACAACCT AGACCGTGGC TGGAGAGGAA GTACGGATAT TACCATCCAA
 GGAGGCGATG ACGTATTCAA AGAGAATTAC GTTACGCTAT TGGGTACCTT 2700
 TGATGAGTGC TATCCAACGT ATTTATATCA AAAAATAGAT GAGTCGAAAT
 TAAAAGCCTA TACCCGTTAT CAATTAAGAG GGTATATCGA AGATAGTCAA 2800
 GACTTAGAAA TCTATTTAAT TCGCTACAAT GCAAAACATG AAACAGTAAA
 TGTGCCAGGT ACGGGTTTCT TATGGCCGCT TTCAGCCCAA AGTCCAATCG 2900
 GAAAGTGTGG AGAGCCGAAT CGATGCGCGC CACACCTTGA ATGGAATCCT
 GACTTAGATT GTTCGTGTAG GGATGGAGAA AAGTGTGCCC ATCATTCGCA 3000
 TCATTTCTCC TTAGACATTG ATGTAGGATG TACAGACTTA AATGAGGACC
 TAGGTGTATG GGTGATCTTT AAGATTAAGA CGCAAGATGG GCACGCAAGA 3100
 CTAGCGAATC TAGAGTTTCT CGAAGAGAAA CCATTAGTAG GAGAAGCGCT
 AGCTCGTGTG AAAAGAGCGG AGAAAAAATG GAGAGACAAA CBTGAAAAAT 3200
 TGGAAATGGGA AACAAATATC GTTTATAAAG AGGCAAAAGA ATCTGTAGAT
 GCTTTATTTG TAAACTCTCA ATATGATCAA TTACAAGCGG ATACGAATAT 3300
 TGCCATGATT CATGCGGCAG ATAAACGTGT TCATAGCATT CGAGAAGCTT
 ATCTGCCTGA GCTGTCTGTG ATTCCGCGTG TCAATGCGGC TATTTTTBAA 3400
 GAATTAGAAG GGCATATTTT CACTGCATTG TCCCTATATG ATGCGAGAAA
 TGTCAATAAA AATGGTGATT TTAATAATGG CTTATCCTGC TGGAAAGTGA 3500
 AAGGGCATGT AGATGTAGAA GAACAAAACA ACCAACGTTT GGTCTTTGTT
 CTTCCGGAAT GGGAAGCAGA AGTGTCAACA GAAGTTCGTG TCTGTCCGGG 3600
 TCGTGGCTAT ATCCTTCGTG TCACAGCGTA CAAGGAGGGA TATGGAGAG
 GTTGCCTAAC CATTGATGAG ATCGAGAACA ATACAGACGA ACTGAAGTTT 3700
 AGCAACTGCG TASAAGAGGA AATCTATCCA AATAACACGG TAACGTGTAA
 TGATTATACT GTAAATCAAG AAGAATACGG AGGTGCGTAC ACTTCTCGTA 3800
 ATCGAGGATA TAACGAAGCT CCTTCCGTAC CAGCTGATTA TGCSTCAGTC
 TATGAAGAAA AATCGTATAC AGATGGACGA AGAGAGAATC CTTGTGAATT 3900
 TAACAGAGGG TATAGGGATT ACACGCCACT ACCAGTTGGT TATGTGACAA
 AAGAAATTAGA ATACTTCCCA GAAACCGATA AGSTATGGAT TGAGATTGGA 4000
 GAAACGGAGG GAACATTTAT CGTGGACAGC GTGGAATTAC TCCTTATGGA
 GGAA (end HD-1)

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Table 3A

Deduced Amino Acid Sequence of Chimeric Toxin

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ACB-1

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MDNNPNIN ECIPYNC LSNFEVEVLGG ER IE
TGYTFIDISLSLTQFLLSEFVFGAGFVLGL
VDI IWGIFGPSQWDAFLVQIEQLINQRIEE
FARNQAISRLEGLSNLYQIYAESFREWEAD
PTNPALREEMRIQFNDMNSALTTAIPLFAV
QNYQVPLLSVYVQAANLHLSVLRDVSVFGQ
RWGFDAAATINSRYNDLTRLIGNYT DYAVRW
YNTGLERVWGPDSRDWVRYNQFRRELTLTV
LDIVALFFPNYDSRRYP IRTVSQLTREIYTN
PVLENFDGSGFRGSAQGIERSIRSPHLM DIL
NSIT IYTD AHRGY YW SGHQIMASPVGFGSG
PEFTFPLYGTMGNAAPQQRIVAQ LGQGVYR
TSSSTLYRRFPFNIGINNQQLSVL DGT EFAY
GTSSSNLPSAVYRKSGTVD SLNEIPPQNNNV
PPRQEF SHRLSHVSMFRSGFSNS SVS IIRA
PTFSWQHRS AEFNNIIFSSQITQIP LTKST
NLGSGT SVVKGP GFTGGDILRR TSPGGQIST
LRVNITAPLSQRYRVRI RYASTTNLQFHTS
IDGRF INQGNFSATMSSSGSNLQSGSFR TVG
FTTPFNFSNGSSVFTLSAHVFNSGNEVYID
RIEFVFAEVTFEAEYDLERAQKAVNELFTS
SNQIGLKT DVTDYHIDQLVSNLVECL SDFC
LDEKQELSEKVKHAKRLSDERNLLQDPNFR
GINROLDRGWRGSTDITIQGGDDVFKENYV
TLLGT FDECYPTYLYQK IDESKLKAYTRYQ
LRGYIEDSQBLEIYLIRYN AKHETVNVPGT
GSLWPLSAQSFI GKCGEPNRCAPHLEWNP D
LDCSCRDGEKCAHHS HHFSLDIDVGC TDLN
EDLG VWVIFKIKTQDGHARLG NLEFLEEK F
LVGEALARVKRAEKKWRDKREKLEWETNIV
YKEAKESVDALFVNSQYDQLQADTNIA MIH
AADKRVHSIREAYLP ELSVIPGVNA AIFEE
LEGRIFTAFSLYDARNV IKN GDFNNGLS CW
NVKGHV DVEEQNNQR SVLVLP EW EA EVSQE
VVRVCPGRGYILRV TAYKEGYGEGCV TIHEI
ENNNTDELKFSNCVEEEIYPNNTVTCNDYTV
NQEEYGGAYTSRN RGYNEAPSVPADYASVY
EEKSYTDGRRENPC EFN RGYRDTPLPVGY
VTKELEYFPETDKVWIEIGETEGT FIVDSV
ELLLMEE

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Table 4

Nucleotide Sequence of Plasmid pSYW1 Encoding
Chimeric Toxin SYW1

The nucleotide differences as compared to the sequence shown in Table 1 are underlined at positions 1252, 1319, 1320, 1323, 1324, and 1326; and code for amino acid changes at positions 289, 311, and 313, as shown in Table 4A.

		(start HD-73)	ATG GATAACAATC 400
	CGAACATCAA	TGAATGCATT CCTTATAATT	GTTTAAGTAA CCCTGAAGTA
20	GAAGTATTAG	GTGGAGAAAG AATAGAAACT	GGTTACACCC CAATCSATAT 500
	TTCTTTGTCT	CTAACGCAAT TTCTTTTGTG	TGAATTTGTT CCCGGTGTCT
	GATTTGTGTT	AGGACTAGTT GATATAATAT	GGGGAATTTT TEGTCCCTCT 600
	CAATGGGACG	CATTTCTTGT ACAAATTGAA	CAGTTAATTA ACCAAAGAAT
	AGAAGAATTC	GCTAGGAACC AAGCCATTTT	TAGATTAGAA GGACTAAGCA 700
25	ATCTTTATCA	AATTTACGCA GAATCTTTTA	GAGAGTGGGA AGCAGATCCT
	ACTAATCCAG	CATTAAGAGA AGAGATGCCT	ATTCATTCA ATGACATGAA 800
	CAGTSCCCTT	ACAACCGCTA TTCCTCTTTT	TGCAGTTCAA AATTATCAAG
	TTCTCTTTT	ATCAGTATAT GTTCAAGCTG	CAAATTTACA TTTATCAGTT 900
	TTGAGAGATG	TTTCAGTGTT TGGACAAAGG	TGGGGATTTG ATGCCGCGAC
30	TATCAATAGT	CGTTATAATG ATTTAACTAG	GCTTATTGGC AACTATACAG 1000
	ATTATGCTGT	ACGCTGGTAC AATACGGGAT	TAGAACGTGT ATGGGGACCG
	GATTCTAGAG	ATTGGGTAAG GTATAATCAA	TTTAGAAGAG AATTAACACT 1100
	AACTGTATTA	GATATCGTTG CTCTGTTCCC	GAATTATGAT AGTAGAAGAT
	ATCCAATTCG	AACAGTTTCC CAATTAACAA	GAGAAATTTA TACAAACCCA 1200
35	GTATTAGAAA	ATTTTGATGG TAGTTTTGCA	GGCTCGGCTC AGGGCATAGA
	AGGAAGTATT	AGGAGTCCAC ATTTGATGGA	TATACTTAAC AGTATAACCA 1300
	TCTATACGSA	TGCTCATAA <u>GGGGAATATT</u>	ATTGGTCAGG GCATCAAATA
	ATGGCTTCTC	CTGTAGGGTT <u>TTGGGGGCCA</u>	GAATTCACCT TTCCGCTATA 1400
	TGGAACATAT	GGAAATGCAG CTCCACAACA	ACGTATTGTT GCTCAACTAG
40	GTGAGGGCGT	GTATAGAACA TTATCGTCCA	CTTTATATAG AAGACCTTTT 1500
	AATATAGGGA	TAAATAATCA ACAACTATCT	GTTCTTGACG GGACAGAAAT
	TGCTTATGSA	ACCTCCTCAA ATTTGCCATC	CGCTGTATAC AGAAAAAGCG 1600
	GAACGGTAGA	TTGCTGGSAT GAAATACCGC	CACAGAATAA CAACGTGCCA
	CCTAGGCAAG	GATTTAGTCA TCSATTAAGC	CATGTTTCAA TGTTTCGTTT 1700
45	AGGCTTIAST	AATAGTAGTG TAAGTATAAT	AAGAGCT (end hd-73)
	(start HD-1)	CCAACGT TTTCTTGCCA	GCATCGCACT 1900
	GCTGAATTTA	ATAATATAAT TCCTTCATCA	CAAATTACAC AAATACCTTT
	AACAAAATCT	ACTAATCTTG GCTCTGGAAC	TTCTGTCTGT AAAGGACCGA 2000
	GATTTACAGG	AGGAGATATT CTTGSAAGAA	CTTCACCTGG CCAGATTTCA
	ACCTTAAGAG	TAAATATTAC TGCACCATTA	TCACAAAGAT ATCGGGTAAG 2100
50	AATTCGCTAC	GCTTCTACTA CAAATTTACA	ATTCCATACA TCAATTGACG
	GAAGACCTAT	TAATCAGGGT AATTTTTCAG	CAACTATGAG TAGTGGGAGT 2200
	AATTTACAGT	CCGGAAGCTT TAGGACTGTA	GGTTTTACTA CTCCGTTTAA
	CTTTTCAAAT	GGATCAAGTG TATTTACGTT	AAGTGCTCAT GTCTTCAATT 2300
	CAGGCAATGA	AGTTTATATA GATCGAATTG	AATTTGTTCC GGCAGAAGTA
55	ACCTTTGAGG	CAGAATATGA TTTAGAAAGA	GCACAAAAGG CGGTGAATGA 2400
	GCTGTTTACT	TCTTCCAATC AAATCGGGTT	AAAAACAGAT GTGACGGATT

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Table 4 (cont.)

15 ATCATATTGA TCAAGTATCC AATTTAGTTG AGTGTTTATC AGATGAATTT 2500
 TGTCTGGATG AAAAACAAGA ATTGTCCGAG AAAGTCAAAC ATGCGAAGCG
 ACTTAGTGAT GAGCGGAATT TACTTCAAGA TCCAACTTC AGAGGGATCA 2600
 ATAGACAAC AGACCGTGGC TGGAGAGGAA GTACGGATAT TACCATCCAA
 GGAGGGGATG ACGTATTCAA AGAGAATTAC GTTACGCTAT TGGGTACCTT 2700
 20 TGATGAGTGC TATCCAACGT ATTTATATCA AAAAATAGAT GAGTCGAAAT
 TAAAAGCCTA TACCCGTTAT CAATTAAGAG GGTATATCGA AGATAGTCAA 2800
 GACTTAGAAA TCTATTTAAT TCGCTACAAT GCAAAACATG AAACAGTAAA
 TGTGCCAGGT ACGGGTTCCT TATGGCCGCT TTCAGCCCAA AGTCCAATCG 2900
 GAAAGTGTGG AGAGCCGAAT CGATGCGCGC CACACCTTGA ATGGAATCCT
 25 GACTTAGATT GTTCGTGTAG GGATGGAGAA AAGTGTGCCC ATCATTGCGA 3000
 TCATTTCTCC TTAGACATTG ATGTAGGATG TACAGACTTA AATGAGGACC
 TAGGTGTATG GGTGATCTTT AAGATTAAGA CCGAAGATGG GCACGCAAGA 3100
 CTAGGGAATC TAGAGTTTCT CGAAGAGAAA CCATTAGTAG GAGAAGGCGT
 AGCTCGTGTG AAAAGAGCGG AGAAAAATG GAGAGACAAA CGTGAAAAAT 3200
 30 TGGAAATGGGA AACAAATATC GTTTATAAAG AGGCAAAAGA ATCTGTAGAT
 GCTTTATTTG TAAACTCTCA ATATGATCAA TTACAAGCGG ATACGAATAT 3300
 TGCCATGATT CATGCGGCAG ATAAACGTGT TCATAGCATT CGAGAAGGCT
 ATCTGCCTGA GCTGTCTGTG ATTCCGGGTG TCAATGCGGC TATTTTGA 3400
 GAATTAGAAG GGCATTTTT CACTGCATTC TCCCTATATG ATGCGAGAAA
 35 TGTCATTAAA AATGGTGATT TTAATAATGG CTTATCCTGC TGAACGTGA 3500
 AAGGGCATGT AGATGTAGAA GAACAAAACA ACCAACGTTT GGTCTTGT
 CTTCCGGAAT GGGAGGAGAA AGTGTACAAA GAAGTTCGTG TCTGTCCGGG 3600
 TCGTGGCTAT ATCCTTCGTG TCACAGCGTA CAAGGAGGGA TATGGAGAAG
 GTTGCSTAAC CATTATGAG ATCGAGAACA ATACAGACGA ACTGAAGTTT 3700
 40 AGCAACTGCG TAGAAGAGGA AATCTATCCA AATAACACGG TAACGTGTAA
 TGATTATACT GTAAATCAAG AAGAATACGG AGGTGCGTAC ACTTCTCGTA 3800
 ATCGAGGATA TAACGAAGCT CTTTCCGTAC CAGCTGATTA TCGTCACTC
 TATGAAGAAA AATCGTATAC AGATGGACGA AGAGAGAATC CTTGTGAATT 3900
 TAACAGAGGG TATAGGGATT ACACGCCACT ACCAGTTGGT TATGTGACAA
 45 AAGAATTAGA ATACTTCCCA GAAACCGATA AGGTATGGAT TGAGATTGGA 4000
 GAAACGGAAG GAACATTTAT CGTGGACAGC GTGGAATTAC TCCTTATGGA
 GGAA (end HD-1)

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Table 4A
Deduced Amino Acid Sequence of Chimeric Toxin SYW1

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M D N N F N I N E C I F Y N C L S N F E V E V L G G E R I E
T G Y T F I D I S L S L T Q F L L S E F V F G A G F V L G L
V D I I W G I F G P S Q W D A F L V Q I E Q L I N Q R I E E
F A R N Q A I S R L E G L S N L Y Q I Y A E S F R E W E A D
P T N F A L R E E M R I Q F N D M N S A L T T A I P L F A V
Q N Y Q V F L L S V Y V Q A A N L H L S V L R D V S V F G Q
R W G F D A A T I N S R Y N D L T R L I G N Y T D Y A V R W
Y N T G L E R V W G P D S R R Y P I R T V S Q L T R E I Y T N
L D I V A L F P N Y D S R R Y P I R T V S Q L T R E I Y T N
P V L E N F D G S F R G S A Q G I E G S I R S P H L M D I L
N S I T I Y T D A H K G E Y Y W S G H Q I M A S P V G F S G
P E F T F P L Y G T M G N A A P Q Q R I V A Q L G Q G V Y R
T L S S T L Y R R P F N I G I N N Q Q L S V L D G T E F A Y
G T S S N L F S A V Y R K S G T V D S L D E I P P Q N N N V
P P R Q G F S H R L S H V S M F R S G F S N S S V S I I R A
P T F S W Q H R S A E F N N I I F S S Q I T Q I P L T K S T
N L G S G T S V V K G P G F T G G D I L R R T S P G Q I S T
L R V N I T A P L S Q R Y R V R I R Y A S T T N L Q F H T S
I D G R P I N Q G N F S A T M S S G S N L Q S G S F R T V G
F T T P F N F S N G S S V F T L S A H V F N S G N E V Y I D
R I E F V P A E V T F E A E Y D L E R A Q K A V N E L F T S
S N Q I G L K T D V T D Y H I D Q V S N L V E C L S D E F C
L D E K Q E L S E K V K H A K R L S D E R N L L Q D P N F R
G I N R Q L D R G W R G S T D I T I Q G G D D V F K E N Y V
T L L G T F D E C Y P T Y L Y Q K I D E S K L K A Y T R Y Q
L R G Y I E D S Q D L E I Y L I R Y N A K H E T V N V P G T
G S L W F L S A Q S P I G K C G E P N R C A P H L E W N P D
L D C S C R D G E K C A H H S H H F S L D I D V G C T D L N
E D L G V W V I F K I K T Q D G H A R L G N L E F L E E K P
L V G E A L A R V K R A E K K W R D K R E K L E W E T N I V
Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M I H
A A D K R V H S I R E A Y L P E L S V I P G V N A A I F E E
L E G R I F T A F S L Y D A R N V I K N G D F N N G L S C W
N V K G H V D V E E Q N N Q R S V L V L P E W E A E V S Q E
V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H E I
E N N T D E L K F S N C V E E E I Y P N N T V T C N D Y T V
N Q E E Y G G A Y T S R N R G Y N E A F S V F A D Y A S V Y
E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V G Y
V T K E L E Y F P E T D K V W I E I G E T E G T F I V D S V
E L L L M E E

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Claims

1. A process for altering the host range of Bacillus toxins which comprises recombining in vitro the variable region of two or more Bacillus toxin genes.
2. A process, according to claim 1, wherein the Bacillus is a Bacillus thuringiensis.
3. A process, according to claim 2, wherein variable regions of Bacillus thuringiensis var. kurstaki HD-1 and Bacillus thuringiensis var. kurstaki HD-73 are recombined in vitro to give genes encoding chimeric toxins having altered host ranges.
4. DNA, denoted pEW3, encoding a chimeric toxin having pesticidal activity, as follows:

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                (start HD-73)                ATG GATAACAATC 400
CGAACATCAA TGAATGCATT CCTTATAATT GTTTAAGTAA CCCTGAAGTA
GAAGTATTAG GTGGAGAAAG AATAGAAACT GGTTACACCC CAATCGATAT 500
TTCCTTGTCG CTAACGCAAT TTCTTTTGAG TGAATTTGTT CCCGGTGCTG
GATTTGTGTT AGGACTAGTT GATATAATAT GGGGAATTTT TGGTCCCTCT 600
CAATGGGACG CATTTCTTGT ACAAATTGAA CAGTTAATTA ACCAAAGAAT
AGAAGAATTC GCTAGGAACC AAGCCATTTT TAGATTAGAA GGAATAAGCA 700
ATCTTTATCA AATTTACGCA GAATCTTTTA GAGAGTGGGA AGCAGATCCT
ACTAATCCAG CATTAAAGAGA AGAGATGCGT ATTCAATTCA ATGACATGAA 800
CAGTGCCCTT ACAACCGCTA TTCCTCTTTT TGCAGTTCAA AATTATCAAG
TTCCTCTTTT ATCAGTATAT GTTCAAGCTG CAAATTTACA TTTATCAGTT 900
TTGASAGATG TTTCAGTGTT TGGACAAAGG TGGGGATTG ATGCCGCGAC
TATCAATAGT CGTTATAATG ATTTAACTAG GCTTATTGGC AACTATACAG 1000
ATTATGCTGT ACGCTGGTAC AATACGGAT TAGAACGTGT ATGGGGACCG
GATTCTAGAG ATTGGSTAAG GTATAATCAA TTTAGAAGAG AATTAACACT 1100
AACTGTATTA GATATCGTTG CTCTGTTCCC GAATTATGAT AGTAGAAGAT
ATCCAATTCG AACAGTTTCC CAATTAACAA GAGAAATTTA TACAAACCCA 1200
GTATTAGAAA ATTTTGATGG TAGTTTTCGA GGCTCGGCTC AGGGCATAGA
AAGAAGTATT AGGAGTCCAC ATTTGATGGA TATACTTAAC AGTATAACCA 1300
TCTATACGGA TGCTCATAGG GGTTATTATT ATTGGTCAGG GCATCAAATA
ATGGCTTCTC CTGTAGGGTT TTCGGGGCCA GAATTCACCT TTCCGCTATA 1400
TGGAACTATG GGAAATGCAG CTCCACAACA ACGTATTGTT GCTCAACTAG
GTCAGGGCGT GTATAGAACA TTATCGTCCA CTTTATATAG AAGACCTTTT 1500
AATATAGGGA TAAATAATCA ACAACTATCT GTTCTTGACG GGACAGAATT
TGCTTATGGA ACCTCCTCAA ATTTGCCATC CGCTGTATAC AGAAAAAGCG 1600
GAACGGTAGA TTCGCTGGAT GAAATACCGC CACAGAATAA CAACGTGCCA

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CCTAEGCAAG GATTTAGTCA TCGATTAAGC CATGTTTCAA TGTTTCGTTT 1700
 AGGCTTTAGT AATAGTAGTG TAAGTATAAT AAGAGCT (end hd-73)
 (start HD-1) CCAACGT TTTCTTGSCA GCATCGCAGT 1900
 5 GCTGAATTTA ATAATATAAT TCCTTCATCA CAAATTACAC AAATACCTTT
 AACAAAATCT ACTAATCTTG GCTCTGGAAC TTCTGTCGTT AAAGGACCAG 2000
 GATTTACAGG AGGAGATATT CTTCGAAGAA CTTCACCTGG CCAGATTTCA
 ACCTTAAGAG TAAATATTAC TGCACCATT A TCACAAAGAT ATCGG3TAAG 2100
 AATTCGCTAC GCTTCTACTA CAAATTTACA ATTCCATACA TCAATTGACG
 GAAGACCTAT TAATCAGGGT AATTTTTTCAG CAACTATGAG TAGTGGGAGT 2200
 10 AATTTACAGT CCGGAAGCTT TAGGACTGTA GGTTTTACTA CTCCGTTTAA
 CTTTTCAAAT GGATCAAGTG TATTTACGTT AAGTGCTCAT GTCTTCAATT 2300
 CAGGCAATGA AGTTTATATA GATCGAATTG AATTTGTTCC GGCAGAAGTA
 ACCTTTGAGG CAGAATATGA TTTAGAAAGA GCACAAAAGG CGGTGAATGA 2400
 GCTGTTTACT TCTTCCAATC AAATCGGGT AAAAACAGAT GTGACGGATT
 15 ATCATATTGA TCAAGTATCC AATTTAGTTG AGTGTTCATC AGATGAATTT 2500
 TGTCTGGATG AAAACAAGA ATTGTCCGAG AAAGTCAAAC ATGCGAAGCG
 ACTTAGTGAT GAGCGGAATT TACTTCAAGA TCCAACTTC AGAGGGATCA 2600
 ATAGACAAC AGACCGTGGC TGGAGAGSAA GTACGGATAT TACCATCCAA
 GGAGGCGATG ACGTATTCAA AGAGAATTAC GTTACGCTAT TGGGTACCTT 2700
 20 TGATGAGTGC TATCCAACGT ATTTATATCA AAAAATAGAT GAGTCGAAAT
 TAAAAGCCTA TACCCGTTAT CAATTAAGAG GGTATATCGA AGATAGTCAA 2800
 GACTTAGAAA TCTATTTAAT TCGCTACAAT GCAAAACATG AAACAGTAAA
 TGTGCCAGGT ACGGGTTCC TATGGCCGCT TTCAGCCCAA AGTCCAATCG 2900
 GAAAGTGTGG AGAGCCGAAT CGATGCGCGC CACACCTTGA ATGGAATCCT
 25 GACTTAGATT GTTCGTGTAG GGATGGAGAA AAGTGTGCC ATCATTCGCA 3000
 TCATTTCTCC TTAGACATTG ATGTAGGATG TACAGACTTA AATGAGGACC
 TAGGTGTATG GGTGATCTTT AAGATTAAGA CGCAAGATGG GCACGCAAGA 3100
 CTAGGGAATC TAGAGTTTCT CGAAGAGAAA CCATTAGTAG GAGAAGCGCT
 AGCTCGTGTG AAAAGAGCGG AGAAAAAATG GAGAGACAAA CGTGA AAAAT 3200
 30 TGGAATGGGA AACAAATATC GTTTATAAAG AGGCAAAAGA ATCTGTAGAT
 GCTTTATTTG TAAACTCTCA ATATGATCAA TTACAAGCGG ATACGAATAT 3300
 TGCCATGATT CATGCGGCAG ATAAACGTGT TCATAGCATT CGA3AAGCTT
 ATCTGCCTGA GCTGTCTGTG ATTCCGGGTG TCAATGCGGC TATTTTGGAA 3400
 GAATTAGAAG GCGTATTTT CACTGCATTC TCCCTATATG ATGCGAGAAA
 35 TGTCAATAAA AATGGTGATT TTAATAATGG CTTATCCTGC TGGAACGTGA 3500
 AAGGGCATGT AGATGTAGAA GAACAAAACA ACCAACGTTT GGTCTTGT
 CTTCCGGAAT GGGAAAGCAGA AGTGTCACAA GAAGTTCGTG TCTGTCCGGG 3600
 TCGTGGCTAT ATCCTTCGTG TCACAGCGTA CAAGGAGGGA TATGAGAGG
 GTTGCCTAAC CATTATGAG ATCGAGAACA ATACAGACGA ACTGAAGTTT 3700
 AGCAACTGCG TAGAAGAGGA AATCTATCCA AATAACACGG TAACGTGTAA
 40 TGATTATACT GTAAATCAAG AAGAATACGG AGGTGCGTAC ACTTCTCGTA 3800
 ATCGAGGATA TAACGAAGCT CTTCCGTAC CAGCTGATTA TCGCTCAGTC
 TATGAAGAAA AATCGTATAC AGATGGACGA AGAGAGAATC CTTGTGAATT 3900
 TAACAGAGGG TATAGGGATT ACACGCCACT ACCAGTTGGT TATGTGACAA
 AAGAATTAGA ATACTTCCCA GAAACCGATA AGGTATGGAT TGAGATTGGA 4000
 45 GAACCGGAAG GAACATTTAT CGTGGACAGC GTGGAATTAC TCCTTATGGA
 GGAA (end HD-1)

and equivalent nucleotide sequences coding for toxin EW3 with the following amino acid sequence:

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NDNNFNINECIFYNCLSNFEVEVVLGGGERIE
TG YTFIDISLSLTQFLLVQIEQLINQRIEES
VDIINGIFGFSQWDAFLVQIEQLINQRIEES
FARNQAISRLEGLSNLYQIYAESFREWEAD
FTNPAALREEMRIQFNDMNSALTTAIFLFAV
QNYQVPLLSVYVQAANLHLSVLRDVSVFGQ
RWGFDAAATINSRYNDLTRLIGNYTDYAVRW
YNTGLERVWGPDSRRYFIRTVSQLTREIYTN
LDIVALLFPNYDSRSGSAOGIERSIASFHLMDIL
PVLLENFDDGSGFRGSAOGIERSIASFHLMDIL
NSTITTYTDAHRGYYWSSHQIMASAFVGFSS
PEPTTFLYGTMGNAAPQORIVAGLGVVYR
TLSSITLYRRFFNIGINNQQLSVLDGTEFAY
GTSSSNLPSAVYRKSGTVDSSLDEIFPNNNV
PFRQGFSHRLSHVSMFRSGGFSNSSSVSIRA
PTFSWQHRSAEFNNIIFSSQITQIPLTKST
NLGSGTSSVVKGGFTGGDILRRRTSPGGIST
LRVNIITAPLSQRYRVRIRYASTTNLQFHTS
IDGRFINQGNFSATMSSSGSNLQSGSFRTVG
FTTFFNFNSNGSSSVFTLSAHVFNSGNEVYID
RIEFVPAEVTFEAEYDLERAGQKAVNELFTS
SNQIGLKTDTVTDYHIDQVSNLVECLSDFC
LDEKQELSEKVKHAKRLSDERNLLQDPNFR
GINRQLDRGWRSSTDITIQGGDDVFKENYV
TLLGTFFDECYPTYLYQKIDESKCLKAYTRYQ
LRGYIEDSQDLEIYLYRYNACKHETVNVPGT
GSLWFLSAQSFISGKCGEFPNRCAPHLEWNPQ
LDSCSCRDGGEKCAHSHHFSLDIDVGCIDLN
EDLGVWVIFKIKTQDGHARLGNLEFLEEKPF
LVGEALARVKRAEKKWRDKREKLEWETNIV
YKEAKESVDALFVNSOYDQADTNIAMIH
AADKRVHSIREAYLPELSVIFGVNAAI FEE
LEGRIFTAFSLYDARNV IKNGBDFNNGLSCW
NVKGHVDEVEEQNNQRSVLVLPFEWEAEVSOE
VRVCPGRGYILRVTA YKEGYGEGCVTIHEI
ENNTDELKFSNCVEEEIYFNNTVT CNDYTV
NQEEYGGAYTSRNRGYNEAFSVPADYASVY
EEKSYTDGRRENFCFNRGYRDTPLFVGY
VTKELEYFPETDKVWIEIGETEGTFIVDSV
ELL L MEE.
  
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5. DNA, denoted pEW4, encoding a chimeric toxin, having pesticidal activity, as follows:

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      (start HD-1)          ATGG ATAACAATCC GAACATCAAT
GAATGCATTG CTTATAATTG TTTAAGTAAC CCTGAAGTAG AAGTATTAGS 600
TGGAGAAAGA ATAGAAACTG GTTACACCCC AATCGATATT TCCTTGTCGC
10 TAACGCAATT TCTTTTGAGT GAATTTGTTT CCGGTGCTGG ATTTGTGTTA 700
GGACTAGTTG ATATAATATG GGGAAATTTT GGTCCCTCTC AATGSSACGC
ATTTCTGTGA CAAATTGAAC AGTTAATTAA CCAAAGAATA GAAGAATTCTG 800
CTAGGAACCA AGCCATTTCT AGATTAGAAG GACTAAGCAA TCTTTATCAA
ATTTACGCAG AATCTTTTAG AGAGTGGGAA GCAGATCCTA CTAATCCAGC 900
15 ATTAAGAGAA GAGATGCGTA TTCAATTCAA TGACATGAAC AGTGCCCTTA
CAACCGCTAT TCCTCTTTTG GCAGTTCAAA ATTATCAAGT TCCTCTTTTA 1000
TCAGTATATG TTCAAGCTGC AAATTTACAT TTATCAGTTT TGAGAGATGT
TTCAGTGTGT GGACAAAGGT GGGGATTTGA TGCCGCGACT ATCAATAGTC 1100
GTTATAATGA TTTAACTAGG CTTATTGGCA ACTATACAGA TTATGCTGTG
20 CGCTGGTACA ATACGGGATT AGAGCGTGTA TGGGGACCGG ATTCTAGAGA 1200
TTGGGTAAGG TATAATCAAT TTAGAAGAGA GCTAACACTT ACTGTATTAG
ATATCGTTGC TCTATTCTCA AATTATGATA GTCGAAGSTA TCCAATTCGA 1300
ACAGTTTCCC AATTAACAAG AGAAATTTAT ACGAACCAGG TATTAGAAAA
TTTTGATGGT AGTTTTCGTG GAATGGCTCA GAGAATAGAA CAGAATATTA 1400
25 GGCAACCACA TCTTATGGAT ATCCTTAATA GTATAACCAT TTATACTGAT
GTGCATAGAG GCTTTAATTA TTGGTCAGGG CATCAAATAA CAGCTTCTCC 1500
TGTAGGGTTT TCAGGACCGA AATTCGCATT CCCTTTATTT GGGAAATGCGG
GGAAATGCAG TCCACCCGTA CTTGTCTCAT TAAGTGGTTT GGGGATTTTT 1600
AGAACATTAT CTTACCTTTT ATATAGAAGA ATTATACTTG GTTCAGGCCC
30 AAATAATCAG GAACTGTTTG TCCTTGATGG AACGGAGTTT TCTTTTGCCT 1700
CCCTAACGAC CAACTTGCCCT TCCACTATAT ATAGACAAAG GGGTACAGTC
GATTCACTAG ATGTAATACC GCCACAGGAT AATAGTGTAC CACCTCGTGC 1800
GGGATTTAGC CATCGATTGA GTCATGTTAC AATGCTGAGC CAAGCAGCTG
GAGCAGTTTA CACCTTGAGA GCTCAACGT (stop HD-1)
      (start HD-73)          CCT ATGTTCTCTT
35 GSATACATCG TAGTGCTGAA TTTAATAATA TAATTGCATC GGATAGTATT 1800
ACTCAAATCC CTGCAGTGAA GGGAACTTT CTTTTTAATG GTTCTGTAAT
TTCAGSACCA GSATTTACTG GTGGGGACTT AGTTAGATTA AATAGTAGTG 1900
GAAATAACAT TCAGAATAGA GGGTATATTG AAGTTCCAAT TCACTTCCCA
TCGACATCTA CCAGATATCG AGTTCGTGTA CGGTATGCTT CTGTAACCCC 2000
40 GATTCACCTC AACGTTAATT GGGGTAATTC ATCCATTTTT TCCAATACAG
TACCAGCTAC AGCTACGTCA TTAGATAATC TACAATCAAG TGATTTTGGT 2100
TATTTTGAAA GTGCCAATGC TTTTACATCT TCATTAGGTA ATATAGTAGG
TGTTAGAAAT TTTAGTGGGA CTGCAGGAGT GATAATAGAC AGATTTGAAT 2200
TTATTCCAGT TACTGCAACA CTCGAGGCTG AATATAATCT GGAAAGAGCG
45 CAGAAGGCGG TGAATGCGCT GTTTACGTCT ACAAACCAAC TAGGGCTAAA 2300
AACAAATGTA ACGGATTATC ATATTGATCA AGTGTTCAAT TTAGTTACGT
ATTTATCGGA TGAATTTTGT CTGGATGAAA AGCGAGAATT GTCCGAGAAA 2400
GTCAAACATG CGAAGCGACT CAGTGATGAA CGCAATTTAC TCCAAGATTC
AAATTTCAAA GACATTAATA GGCAACCAGA ACGTGGGTGG GSCGGAAGTA 2500
50 CAGGGATTAC CATCCAAGGA GGGGATGACG TATTTAAAGA AAATTACGTC
ACACTATCAG GTACCTTTGA TGAGTGCTAT CCAACATATT TGTATCAAAA 2600
AATCGATGAA TCAAAATTAA AAGCCTTTAC CCGTTATCAA TTAAGAGGGT
ATATCGAAGA TAGTCAAGAC TTAGAAATCT ATTTAATTCT CTACAATGCA 2700
AAACATGAAA CAGTAAATGT GCCAGGTACG GGTTCCTTAT GGCCGCTTTC

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	AGCCCAAAGT	CCAATCGGAA	AGTGTGGAGA	GCCGAATCGA	TGCGCGCCAC	2200
	ACCTTGAATG	GAATCCTGAC	TTAGATTGTT	CGTGTAGGGA	TGGAGAAAAG	
	TGTGCCCATC	ATTCGCATCA	TTTCTCCTTA	GACATTGATG	TAGSATGTAC	2900
5	AGACTTAAAT	GAGGACCTAG	GTGTATGGST	GATCTTTAAG	ATTAAGACGC	
	AAGATGGGCA	CGCAAGACTA	GGGAATCTAG	AGTTTCTCGA	AGAGAAACCA	3000
	TTAGTAGGAG	AAGCGCTAGC	TCGTGTGAAA	AGAGCGGAGA	AAAAATGGAG	
	AGACAAACGT	GAAAAATTGG	AATGGGAAAC	AAATATCGTT	TATAAAGAGG	3100
	CAAAAGAATC	TGTAGATGCT	TTATTTGTAA	ACTCTCAATA	TGATCAATTA	
10	CAAGCGGATA	CGAATATTGC	CATGATTCAT	GCGGCAGATA	AACGTGTTCA	3200
	TAGCATTCSA	GAAGCTTATC	TGCCTGAGCT	GTCTGTGATT	CCGGGTGTCA	
	ATGCGGCTAT	TTTTGAAGAA	TTAGAAGGGC	GTATTTTCAC	TGCATTCTCC	3300
	CTATATGATG	CGAGAAATGT	CATTAAAAAT	GGTGATTTTA	ATAATGGCTT	
	ATCCTGCTGG	AACGTGAAAG	GGCATGTAGA	TGTAGAAGAA	CAAAACAACC	3400
	AACGTTCSGT	CCTTGTTGTT	CCGGAATGGG	AAGCAGAAGT	GTCACAAGAA	
15	GTTCTGTGCT	GTCCGGGTCS	TGGCTATATC	CTTCGTGTCA	CAGCGTACAA	3500
	GGAGGGATAT	GGAGAAAGTT	GCGTAACCAT	TCATGAGATC	GAGAACATA	
	CAGACGAACT	GAAGTTTAGC	AACTGCGTAG	AAGAGGAAAT	CTATCCAAAT	3600
	AACACGGTAA	CGTGTAATGA	TTATACTGTA	AATCAAGAAG	AATACGGAGG	
20	TGCGTACACT	TCTCGTAATC	GAGGATATAA	CGAAGCTCCT	TCCGTACCAG	3700
	CTGATTATGC	GTCAGTCTAT	GAAGAAAAAT	CGTATACAGA	TGGACGAAGA	
	GAGAATCCTT	GTGAATTTAA	CAGAGGGTAT	AGGGATTACA	CGCCACTACC	3800
	AGTTGGTTAT	GTGACAAAAG	AATTAGAATA	CTTCCCAGAA	ACCGATAAGG	
	TATGGATTGA	GATTGGAGAA	ACGGAAGGAA	CATTTATCGT	GSACAGCGTG	3900
	GAATTACTCC	TTATGGAGGA	A (end HU-73)			

25 and equivalent nucleotide sequences coding for toxin EW4 with the following amino acid sequence:

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MDNNFPNIN ECIFYNCLSNPEVEVLGGGERIE
 TGYTFIDISLSLTQFLFLSEFVPGAGFVLGL
 VDIINGIIFGPSQWQAFPVQIEQLINQRIEE
 FARNDQAI SRLEGLSNLYQIYAESFREWEAD
 FTNPALREEMRIQFNDMNSALTTAIFLLAV
 QNYQVFLLSVYVQAANLHLSVLRDVSVFGQ
 RWGFQAATINSRYNDLTRLIGNYT DYAVRW
 YNTGLERVWGFDSRDWVRYNQFRRELTLTV
 LDIVALF SNYDSRRYP IRTVSQLTREIYTN
 PVLENFDGSGFRGMAQR IEQNIROPHLM DIL
 NSITIIYTDVHRGFNYWSGHQITASPVGFGS
 PEFAPFLFGNAGNAAPPV LVS LTGLGIFST
 LSSFLYRRRIILGSGFPNNQELFVL DGTFS
 ASLT TNLPSTIYRQRGT VDSLDVIPPQDNS
 VFFRAGF SHRLSHVTMLSQAAGAVYTLRAQ
 RPFMF SWIHRSAEFNNIIASDSITQIPAVKG
 NFLFNGSVISGPGFTGGDLVRLNSSGNNIQ
 NRGYIEVP IHF PSTSTRYRVRVRYASVTFI
 HLN VNWNWGNSSIFSNTPATATSLDNLQSSD
 FGYFESANAF TSSSLGNI VGVNFGSGTAGVI
 IDRFEFIPVTATLEAEYNLERAQKAVNALF
 TSTNQ LGLKTNVT D YHIDQVSNLV TYLSDE
 FCLDEKRELSEKVKHAKRLSDERNLLQDSN
 FKDINRQPERGEWGGSTGITIQGGDDVFKEN
 YVTL SGT FDECYPTYLYQK IDESKLKAFTR
 YQLRGYIEDS QDLEIYLIRYN AKHETVNVF
 GTGSLWFLSAQSP I GKC GFENRCAPHLEWN
 PDLDC SCRDGEKCAHHS HHFSLDIDVGCTD
 LNEB LGVWVIFKIKTQDGHARLG NLEFLEE
 KPLVGEALARVKRAEKKWRDKREKADTNIAM
 IVYKEAKESVDALFVNSQYDQSVIFGVNA AIF
 IHAADKRVHSIREAYLFELSVIKNGDFNGLS
 EELEGRIFTAFSLYDARNVIVVPEWEAEVVS
 CWNVKGHV DVEEQNNQRSVLVPEWEGCVTIH
 QEV RVCPGRGYILRV TAYKEGYGEGCVTIH
 EIE NNNTDELKFSNCVEE E IYPNNTVT CNDY
 TVNQEEYGGAYTSRNRGYNEAPSVPADYAS
 VYEEKSYTDGRRENPCFENRGYRDYTPLPV
 GYVTKELEYFPETDKVWIEIGETEGTFIVD
 SVELLLMEE.

6. DNA, denoted pACB-1, encoding a chimeric toxin, having pesticidal activity, as follows:

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5 (start HD-73) ATG GATAACAATC 400
CGAACATCAA TGAATGCATT CCTTATAATT GTTTAAGTAA CCCTGAAGTA
GAAGTATTAG GTGGAGAAAG AATAGAACT GGTACACCC CAATCGATAT 500
TTCTTGTCTG CTAACGCAAT TTCTTTTGAG TGAATTTGTT CCCGCTGCTG
10 GATTTGTGTT AGGACTAGTT GATATAATAT GGGGAATTTT TGGTCCCTCT 600
CAATGGGACG CATTCTTGT ACAAATTGAA CAGTTAATTA ACCAAAGAAT
AGAAGAATTC GCTAGGAACC AAGCCATTTT TAGATTAGAA GGAATAAGCA 700
ATCTTTATCA AATTTACGCA GAATCTTTTA GAGAGTGGGA AGCAGATCCT
ACTAATCCAG CATTAAAGAGA AGAGATGCGT ATTCAATTCA ATGACATGAA 800
CASTGCCCTT ACAACCGCTA TTCCTCTTTT TGCAGTTCAA AATTATCAAG
15 TTCCTCTTTT ATCAGTATAT GTTCAAGCTG CAAATTTACA TTTATCAGTT 900
TTGAGAGATG TTTCAAGTGT TGGACAAAGG TGGGGATTTG ATGCCGCGAC
TATCAATAGT CGTTATAATG ATTTAACTAG GCTTATTGGC AACTATACAG 1000
ATTATGCTGT ACGCTGGTAC AATACGGGAT TAGAACGTGT ATGGGGACCG
GATTCTAGAG ATTGGGTAAG GTATAATCAA TTTAGAAGAG AATTAACACT 1100
20 AACTGTATTA GATATCGTTG CTCTGTTCCC GAATTATGAT AGTAGAAGAT
ATCCAATTCG AACAGTTTCC CAATTAACAA GAGAAATTTA TACAAACCCA 1200
GTATTAGAAA ATTTTGATGG TAGTTTTCGA GGCTCGGCTC AGGGCATAGA
AAGAAGTATT AGGAGTCCAC ATTTGATGGA TATACTTAAC AGTATAACCA 1300
TCTATACGGA TGCTCATAGG GGTTATTATT ATTGGTCAGG GCATCAAATA
25 ATGGCTTCTC CTGTAGGGTT TTCGGGGCCA GAATTCACCT TTCCGCTATA 1400
TGGAACATG GGAATGCAG CTCCACAACA ACGTATTGTT GCTCAACTAG
GTCAGGECST GTATAGAACA TTATCGTCCA CTTTATATAG AAGACCTTTT 1500
AATATAGGGA TAAATAATCA ACAACTATCT GTTCTTGACG GGACAGAATT
TGCTTATGGA ACCTCCTCAA ATTTGCCATC CGCTGTATAC AGAAAAAGCG 1600
30 GAACGSTATG TTCGCTGAAT GAAATACCGC CACAGAATAA CAACGTGCCA
CCTAGGCAAG AATTTAGTCA TCGATTAAAG CATGTTTCAA TGTTCGTTT 1700
AGSCTTTAGT AATAGTAGTG TAAGTATAAT AAGAGCT (end hd-73)
(start HD-1) CCAACGT TTTCTTGSCA GCATCGCAGT 1900
GCTGAATTTA ATAATATAAT TCCTTCATCA CAAATTACAC AAATACCTTT
35 AACAAAATCT ACTAATCTTG GCTCTGGAAC TTCTGTCTGT AAAGGACCAG 2000
GATTTACAGG AGGAGATATT CTTGGAAGAA CTTACCTGG CCASATTTCA
ACCTTAAGAG TAAATATTAC TGCACCATTA TCACAAAGAT ATCGGCTAAG 2100
AATTCGCTAC GCTTCTACTA CAAATTTACA ATTCCATACA TCAATTGAGG
GAAGACCTAT TAATCAGGGT AATTTTTCAG CAACTATGAG TAGTGGGAGT 2200
40 AATTTACAGT CCGGAAGCTT TAGGACTGTA GGTTTTACTA CTCCGTTTAA
CTTTTCAAAT GGATCAAGTG TATTTACGTT AAGTGCTCAT GTCTTCAATT 2300
CAGGCAATGA AGTTTATATA GATCGAATTG AATTTGTTCC GGCAGAAGTA
ACCTTTGAGG CAGAATATGA TTTAGAAAGA GCACAAAAGG CGGTGAATGA 2400
GCTGTTTACT TCTTCCAATC AAATCGGGTT AAAACAGAT GTGACGGATT
ATCATATTGA TCAAGTATCC AATTTAGTTG AGTGTTTATC AGATGAATTT 2500
45 TGTCTGATG AAAACAAGA ATTGTCCGAG AAAGTCAAAC ATGCGAAGCG
ACTTAGTGAT GAGCGGAATT TACTTCAAGA TCCAAACTTC AGAGGGATCA 2600
ATAGACAACCT AGACCGTGGC TGGAGAGGAA GTACGGATAT TACCATCCAA
GGAGGCGATG ACGTATTCAA AGAGAATTAC GTTACGCTAT TGGGTACCTT 2700
TGATGAGTGC TATCCAACGT ATTTATATCA AAAAATAGAT GAGTCGAAAT
50 TAAAAGCCTA TACCCGTTAT CAATTAAGAG GGTATATCGA AGATAGTCAA 2800
GACTTAGAAA TCTATTTAAT TCGCTACAAT GCAAAACATG AAACAGTAAA
TGTGCCAGGT ACGGGTTCCCT TATGGCCGCT TTCAGCCCAA AGTCCAATCG 2900
GAAAGTGTTG AGAGCCGAAT CGATGCGCGC CACACCTTGA ATGGAATCCT
GACTTAGATT GTTCGTGTAG GGATGGAGAA AAGTGTGCCC ATCATTGCGA 3000
55 TCATTTCTCC TTAGACATTG ATGTAGGATG TACAGACTTA AATGAGGACC

TAGGTGTATG GGTGATCTTT AAGATTAAGA CGCAAGATGG GCACGCAAGA 3100
 CTAGGGAATC TAGAGTTTCT CGAAGAGAAA CCATTAGTAG GAGAAGCGCT
 AGCTCGTGTG AAAAGAGCGG AGAAAAAATG GAGAGACAAA CGTGAAAAAT 3200
 TSSAATGGGA AACAAATATC GTTTATAAAG AGGCAAAAAG ATCTGTAGAT
 5 GCTTTATTTG TAAAGTCTCA ATATGATCAA TTACAAGCGG ATACGAATAT 3300
 TGCCATGATT CATGCGGCAG ATAAACGTGT TCATAGCATT CGAGAAGCTT
 ATCTGCCTGA GCTGTCTGTG ATTCCGGGTG TCAATGCGGC TATTTTGTAA 3400
 GAATTAGAAG GSCGTATTTT CACTGCATTC TCCCTATATG ATGCGAGAAA
 TGTCATTAAA AATGGTGATT TTAATAATGG CTTATCCTGC TGGAACGTGA 3500
 10 AAGGGCATGT AGATGTAGAA GAACAAAACA ACCAACGTTT GGTCTTGTG 3600
 CTTCCGGAAT GGGAAAGCAGA AGTGTACAAA GAAGTTCGTG TCTGTCCGGG
 TCGTGGCTAT ATCCTTCGTG TCACAGCGTA CAAGGAGGGA TATGGAGAAG
 GTTGCGTAAC CATTGATGAG ATCGAGAACA ATACAGACGA ACTGAAGTTT 3700
 AGCAACTGCG TAGAAGAGGA AATCTATCCA AATAACACGG TAACGTGTAA
 15 TGATTATACT GTAAATCAAG AAGAATACGG AGGTGCGTAC ACTTCTCGTA 3800
 ATCGAGGATA TAACGAAGCT CCTTCCGTAC CAGCTGATTA TCGTCACTC
 TATGAAGAAA AATCGTATAC AGATGGACGA AGAGAGAATC CTTGTGAATT 3900
 TAACAGAGGG TATAGGGATT ACACGCCACT ACCAGTTGGT TATGTGACAA
 AAGAATTAGA ATACTTCCCA GAAACCGATA AGGTATGGAT TGAGATTGGA 4000
 20 GAAACGGAAG GAACATTTAT CGTGGACAGC GTGGAATTAC TCCTTATGGA
 GGAA (end HD-1)

and equivalent nucleotide sequences coding for toxin ACB-1 with the following amino acid sequence:

25
 30
 MDNNPNINECIPYNCLSNPEVEVLGGERIE
 TGYTPIDISLSLTQFLLEFVFGAGFVLGL
 VDIIWGIFGPSQWDAFLVQIEQLINQRIEE
 FARNQAIISRLGLESLNLYQIYAESFREWEAD
 35 PTNPALREEMRIQFNDMNSALTTAIPLFAV
 QNYQVPLLSVYVQAANLHLSVLRDVSVFGQ
 RWGFDAATINSRYNDLTRLIGNYT DYAVRW
 YNTGLERVWGFDSRDWVRYNQFRRELT LT V
 LDIVALFFPNYDSRRYFIRTVS QLTREIYTN
 40 PVLENFDGSGFRGSAQGIERSIRSPHLM DIL
 NSITIIYTDHRGYYYWSGHQIMASPVGFSG
 PEFTTFPLYGTMGNAAPQQRIVAQLGQGVYR
 TLSSTLYRRPFNIGINNQQLSVLDGTEFAY
 GTSSNLP SAVYRKSGTVDSLNEIPPQNNNV
 45 PFRQEF SHRLSHVSMFRSGFSNS SVSII RA
 PTFSWQHRS AEFNNIIPSSQITQIPLTKST
 NLGSGT SVVKGP GFTGGDILRRTSPGQIST
 LRVNITAPLSQRYRVRIRYASTTNLQFHTS
 IDGRPINQGNFSATMSSG SNLQSGSFR TVG

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5 F T T P F N F S N G S S V F T L S A H V F N S G N E V Y I D
 R I E F V F A E V T F E A E Y D L E R A Q K A V N E L F T S
 S N Q I G L K T D V T D Y H I D Q V S N L V E C L S D E F C
 L D E K Q E L S E K V K H A K R L S D E R N L L Q D F N F R
 G I N R Q L D R G W R G S T D I T I Q G G D D V F K E N Y V
 T L L G T F D E C Y P T Y L Y Q K I D E S K L K A Y T R Y Q
 L R G Y I E D S Q D L E I Y L I R Y N A K H E T V N V P G T
 G S L W F L S A Q S P I G K C G E F N R C A P H L E W N F D
 10 L D C S C R D G E K C A H H S H H F S L D I D V G C T D L N
 E D L G V W V I F K I K T Q D G H A R L G N L E F L E E K P
 L V G E A L A R V K R A E K K W R D K R E K L E W E T N I V
 Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M I H
 A A D K R V H S I R E A Y L P E L S V I P G V N A A I F E E
 15 L E G R I F T A F S L Y D A R N V I K N G D F N N G L S C W
 N V K G H V D V E E Q N N Q R S V L V L P E W E A E V S Q E
 V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H E I
 E N N T D E L K F S N C V E E E I Y P N N T V T C N D Y T V
 N Q E E Y G G A Y T S R N R G Y N E A P S V P A D Y A S V Y
 20 E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V G Y
 V T K E L E Y F P E T D K V W I E I G E T E G T F I V D S V
 E L L L M E E .

7. DNA, denoted pSYW1, encoding a chimeric toxin, having pesticidal activity, as follows:

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	(start HQ-73)	ATG GATAACAATC 400
CGAACATCAA	TGAATGCATT	CCTTATAATT GTTTAAGTAA CCCTGAAGTA
GAAGTATTAG	GTGGAGAAAG	AATAGAAACT GGTTACACCC CAATCGATAT 500
TTCCTTGTCG	CTAACGCAAT	TTCTTTTGAG TGAATTTGTT CCCGGTGCTG
GATTTGTGTT	AGGACTAGTT	SATATAATAT GGGGAATTTT TGSTCCCTCT 600
CAATGGGACG	CATTTCTTGT	ACAAATTGAA CAGTTAATTA ACCAAGAAT
AGAAGAATTC	GCTAGGAACC	AAGCCATTTT TAGATTAGAA GGACTAAGCA 700
ATCTTTATCA	AATTTACGCA	GAATCTTTTA GAGAGTGGGA AGCAGATCCT
ACTAATCCAG	CATTAAGAGA	AGAGATGCGT ATTCAATTCA ATGACATGAA 800
CAGTGCCCTT	ACAACCGCTA	TTCTCTTTT TGCAGTTCAA AATTATCAAG
TTCTCTTTT	ATCAGTATAT	GTTCAAGCTG CAAATTTACA TTTATCAGTT 900
TTGAGAGATG	TTTCAGTGTT	TGGACAAAGG TGGGGATTTG ATGCCGCGAC
TATCAATAGT	CGTTATAATG	ATTTAACTAG GCTTATTGSC AACTATACAG 1000
ATTATGCTGT	ACGCTGGTAC	AATACGGGAT TAGAACGTGT ATGGGGACCG
GATTCTAGAG	ATTGGGTAAG	GTATAATCAA TTTAGAAGAG AATTAACT 1100
AACTGTATTA	GATATCGTTG	CTCTGTTCCC GAATTATGAT AGTAGAAGAT
ATCCAATTCG	AACAGTTTCC	CAATTAACAA GAGAAATTTA TACAACCCA 1200
GTATTAGAAA	ATTTTGATGG	TAGTTTTCSA GGCTCGGCTC AGGGCATAGA
AGGAAGTATT	AGGAGTCCAC	ATTTGATGGA TATACTTAAC AGTATAACCA 1300
TCTATACGGA	TGCTCATAAA	GGGGAATATT ATTGGTCAGG GCATCAATA
ATGGCTTCTC	CTGTAGGGTT	TTCCGGGCCA GAATTCACCT TTCCGCTATA 1400
TGGAACTATG	GGAAATGCAG	CTCCACAACA ACGTATTGTT GCTCAACTAG
GTCAGGGCGT	GTATAGAACA	TTATCGTCCA CTTTATATAG AAGACCTTTT 1500
AATATAGGSA	TAAATAATCA	ACAATATCT GTTCTTGACG GGACAGAAAT

TGCTTATGSA ACCTCCTCAA ATTTGCCATC CGCTGTATAC AGAAAAAGCG 1600
 GAACGGTAGA TTCGCTGGAT GAAATACCGC CACAGAATAA CAACGTGCCA
 CCTAGGCAAG GATTTAGTCA TCGATTAAGC CATGTTTCAA TGTTCSTTC 1700
 AGGCTTTAGT AATAGTAGTG TAAGTATAAT AAGAGCT (end hd-73)
 5 (start HD-1) CCAACGT TTTCTTGGCA GCATCGCAGT 1900
 GCTGAATTTA ATAATATAAT TCCTTCATCA CAAATTACAC AAATACCTTT
 AACAAAATCT ACTAATCTTG GCTCTGSAAC TTCTGTCTGT AAAGGACCCAG 2000
 GATTTACAGG AGGAGATATT CTTGGAAGAA CTTACCTGG CCAGATTTCA
 ACCTTAAGAG TAAATATTAC TGCACCATTA TCACAAAGAT ATCGGGTAAG 2100
 AATTCGCTAC GCTTCTACTA CAAATTTACA ATTCCATACA TCAATTGACG
 10 GAAGACCTAT TAATCAGGGT AATTTTTCAG CAACTATGAG TAGTGGGAGT 2200
 AATTTACAGT CCGGAAGCTT TAGGACTGTA GGTTTTACTA CTCCGTTTAA
 CTTTTCAAAAT GGATCAAGTG TATTTACGTT AAGTGCTCAT GTCTTCAATT 2300
 CAGGCAATGA AGTTTATATA GATCGAATTG AATTTGTTC GGCAGAAGTA
 ACCTTTGAGG CAGAATATGA TTTAGAAAGA GCACAAAAGG CGGTGAATGA 2400
 15 GCTGTTTACT TCTTCCAATC AAATCGGGTT AAAACAGAT GTGACGGATT
 ATCATATTGA TCAAGTATCC AATTTAGTTG AGTGTTTATC AGATGAATTT 2500
 TGTCTGGATG AAAACAAGA ATTGTCCGAG AAAGTCAAAC ATGCGAAGCG
 ACTTAGTGAT GAGCGGAATT TACTTCAAGA TCCAAACTTC AGAGGGATCA 2600
 ATAGACAACT AGACCGTGGC TGGAGAGGAA GTACGGATAT TACCATCCAA
 20 GGAGGCGATG ACGTATTCAA AGAGAATTAC GTTACGCTAT TGGGTACCTT 2700
 TGATGAGTGC TATCCAACGT ATTTATATCA AAAAATAGAT GAGTCGAAAT
 TAAAAGCCTA TACCCGTTAT CAATTAAGAG GGTATATCGA AGATAGTCAA 2800
 GACTTAGAAA TCTATTTAAT TCGCTACAAT GCAAAACATG AAACAGTAAA
 TGTGCCAGGT ACGGGTTCTT TATGGCCGCT TTCAGCCCAA AGTCCAATCG 2900
 25 GAAAGTGTGG AGAGCCGAAT CGATGCGCGC CACACCTTGA ATGGAATCCT
 GACTTAGATT GTTCGTGTAG GGATGGAGAA AAGTGTGCC ATCATTCGCA 3000
 TCATTTCTCC TTAGACATTG ATGTAGSATG TACAGACTTA AATGAGGACC
 TAGGTGTATG GGTGATCTTT AAGATTAAGA CGCAAGATGG GCACGCAAGA 3100
 CTAGGGAATC TAGAGTTTCT CGAAGAGAAA CCATTAGTAG GAGAGGCGCT
 30 AGCTCGTGTG AAAAGAGCGG AGAAAAAATG GAGAGACAAA CGTGAAAAAT 3200
 TGGAAATGGGA AACAAATATC GTTTATAAAG AGGCAAAAGA ATCTGTAGAT
 GCTTTATTTG TAACTCTCA ATATGATCAA TTACAAGCGG ATACGAATAT 3300
 TGCCATGATT CATGCGGCAG ATAAACGTGT TCATAGCATT CGAGAAGCTT
 ATCTGCCTGA GCTGTCTGTG ATTCCGGGTG TCAATGCGGC TATTTTIGAA 3400
 35 GAATTAGAAAG GGCATTTTT CACTGCATTC TCCCTATATG ATGCGAGAAA
 TGTCATTAAA AATGGTGATT TTAATAATGG CTTATCCTGC TGGAACGTGA 3500
 AAGGGCATGT AGATGTAGAA GAACAAAACA ACCAACGTTT GGTCTTGTG
 CTTCCGGAAT GGGGAAGCAGA AGTGTCACAA GAAGTTCGTG TCTGTCCGGG 3600
 TCGTGGCTAT ATCCTTCGTG TCACAGCGTA CAAGGAGGGA TATGGAGAAG
 40 GTTGCCTAAC CATTATGAG ATCGAGAACAA ATACAGACGA ACTGAAGTTT 3700
 AGCAACTGCG TAGAAGAGGA AATCTATCCA AATAACACGG TAACGTGTAA
 TGATTATACT GTAAATCAAG AAGAATACGG AGGTGCGTAC ACTTCTCGTA 3800
 ATCGAGGATA TAACGAAGCT CCTTCCGTAC CAGCTGATTA TCGTCACTC
 TATGAAGAAA AATCGTATAC AGATGGACGA AGAGAGAATC CTTGTGAATT 3900
 TAACAGAGGG TATAGGGATT ACACGCCACT ACCAGTTGGT TATGTGACAA
 45 AAGAATTAGA ATACTTCCCA GAAACCGATA AGGTATGGAT TGAGATTGGA 4000
 GAAACGGAAG GAACATTTAT CGTGGACAGC GTGGAAATTAC TCCTTATGGA
 GGAA (end HD-1)

and equivalent nucleotide sequences coding for toxin SYW1 with the following amino acid sequence:

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5 M D N N P N I N E C I P Y N C L S N P E V E V L G G E R I E
 T G Y T F I D I S L S L T Q F L L S E F V F G A G F V L G L
 V D I I W G I F G P S Q W D A F L V Q I E Q L I N Q R I E E
 F A R N Q A I S R L E G L S N L Y Q I Y A E S F R E W E A D
 P T N P A L R E E M R I Q F N D M N S A L T T A I P L F A V
 Q N Y Q V P L L S V Y V Q A A N L H L S V L R D V S V F G Q
 R W G F D A A T I N S R Y N D L T R L I G N Y T D Y A V R W
 Y N T G L E R V W G P D S R R Y P I R T V S Q L T R E I Y T N
 10 L D I V A L F P N Y D S R R Y P I R T V S Q L T R E I Y T N
 P V L E N F D G S F R G S A Q G I E G S I R S P H L M D I L
 N S I T I Y T D A H K G E Y Y W S G H Q I M A S P V G F S G
 P E F T F P L Y G T M G N A A P Q Q R I V A Q L G Q G V Y R
 T L S S T L Y R R P F N I G I N N Q Q L S V L D G T E F A Y
 15 G T S S N L P S A V Y R K S G T V D S L D E I P P Q N N N V
 P P R Q G F S H R L S H V S M F R S G F S N S S V S I I R A
 P T F S W Q H R S A E F N N I I P S S Q I T Q I P L T K S T
 N L G S G T S V V K G P G F T G G D I L R R T S P G Q I S T
 L R V N I T A P L S Q R Y R V R I R Y A S T T N L Q F H T S
 20 I D G R P I N Q G N F S A T M S S G S N L Q S G S F R T V G
 F T T P F N F S N G S S V F T L S A H V F N S G N E V Y I D
 R I E F V P A E V T F E A E Y D L E R A Q K A V N E L F T S
 S N Q I G L K T D V T D Y H I D Q V S N L V E C L S D E F C
 L D E K Q E L S E K V K H A K R L S D E R N L L Q D P N F R
 25 G I N R Q L D R G W R G S T D I T I Q G G D D V F K E N Y V
 T L L G T F D E C Y P T Y L Y Q K I D E S K L K A Y T R Y Q
 L R G Y I E D S Q D L E I Y L I R Y N A K H E T V N V P G T
 G S L W P L S A Q S P I G K C G E P N R C A P H L E W N P D
 L D C S C R D G E K C A H S H H F S L D I D V G C T D L N
 30 E D L G V W V I F K I K T Q D G H A R L G N L E F L E E K P
 L V G E A L A R V K R A E K K W R D K R E K L E W E T N I V
 Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M I H
 A A D K R V H S I R E A Y L P E L S V I P G V N A A I F E E
 L E G R I F T A F S L Y D A R N V I K N G D F N N G L S C W
 35 N V K G H V D V E E Q N N Q R S V L V L P E W E A E V S Q E
 V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H E I
 E N N T D E L K F S N C V E E E I Y P N N T V T C N D Y T V
 N Q E E Y G G A Y T S R N R G Y N E A P S V P A D Y A S V Y
 E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V G Y
 40 V T K E L E Y F P E T D K V W I E I G E T E G T F I V D S V
 E L L L M E E .

8. A chimeric toxin, EW3, having pesticidal activity, having the following amino acid sequence:

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5 MDNNFNIN ECIFYNCLSNFEVEVLGGERRIE
 TGYTFIDISLSLTQFLVSEFVPGAGFVLGL
 VDIIWGIFGFSQWDAFLVQIEQLINQRIEE
 FARNQAI SRLEGLSNLYQIYAESFREWEAD
 PTNPALREEMRIQFNNDMNSALTTAIFLFAV
 QNYQVFLLSVYVQAANLHLSVLRDVSVFGQ
 RWGFDAAATINSRYNDLTRLIIGNYT DYAVRW
 YNTGLERVWGPDSRDWVRYNQFRRELTLTV
 10 LDIVALLFPNYDSRRYP IRTVSQLTREIYTN
 PVLENF DGSFRGSAQGIERSIRS FHLMDIL
 NSITIIYTD AHRGYYYWSGHQIMASPVGFGS
 PEFTTFLYGTMGNAAPQQRIVADLGQGVYR
 TSSSTLYRRPFNIGINNQQLSVLDGTEFAY
 15 GTSSSNLPSAVYRKSGTVDSDLDEIFPQNNNV
 FPRGGSF SHRLSHVSMFRSGFSSNSSVSIRRA
 PTFSWQHRS AEFNNIIFSSQITQIFLT KST
 NLGSGT SVVKGP GFTGGDILRRTSPGQIST
 LRVNITAFLSQRYRVRIRYASTTNLQFHTS
 20 IDGRFINQGNFSATMSSSGSNLQSGGSFRTVG
 FTTFFNF SNGSSSVFTLSAHVFNSGNEVYID
 RIEFVFAEVTFEAEYDLERAQKAVNELFTS
 SNQIGLKT DVTDYHIDQVSNLVECLSD EFC
 LDEKQELSEKVKHAKRLSDERNLLQDPNFR
 25 GINRQLDRGWRGSTDITIQGGDDVFKENYV
 TLLGT FDECYPTYLYQK IDESKLKAYTRYQ
 LRGYIEDSDLEIYLIRYN AKHETVNVPGT
 GSLWFLSAQSPIGKCGEPNRCAPHLEWNPD
 LDCSCRDGEKCAHHSHHFSLDIDVGC TDLN
 30 EDLGVWVIFKIKTQDGHARLG NLEFLEEKPF
 LVGEALARVKRAEKKWRDKREKLEWETNIV
 YKEAKESVDALFVNSQYDQLQADTNIA MIH
 AADKR VHSIREAYLP ELSNIFGVNA AIFEE
 LEGRIFTA FSLYDARNVIFNGDFNNGLS CW
 35 NVKGHV DVEEONNORSVLVLPEWEAEVSD E
 VRVCPGRGYILRV TAYKEGYGEGCVTIHEI
 ENNTDELKFSNDVEEEIYFNNTVTCNDYTV
 NQEEYGGAYTSRN RGYNEAPSVPADYASVY
 EEKSYTDGRRENFC EFNRGYRDYTFLPVGY
 40 VTKELEYFPETDKVWIEIGETEGT FIVDSV
 ELLLME E

and mutants thereof which do not alter the protein secondary structure.

9. A chimeric toxin, EW4, having pesticidal activity, having the following amino acid sequence:

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5 MDNNFNIN ECIPYNC LSNPEVEVLGGGERIE
 TGYTFIDISLSLTQFL LSEFVPGAGFVLGL
 VDIINGIFGFSQWDAFPVQIEQLINQRIEE
 FARNQAISRL EGLSNLYQIYAESFREWEAD
 PTNPA LR EEMRIQFNDMNSALT TAIFLLAV
 QNYQVPLLSVYVQAANLHLSVLRDVSVFGG
 RWGFDAATINSRYNDLTRLIGNYT DYA VRW
 YNTGLERVWGFDSRRYP IRTVSQLTRELTIV
 10 LDIVALF SNSYDSRRYPIRTVSQLTRELTIV
 FLVLENF DGSFRRGMARIEQNIROPHLM DIL
 NSITITITDVH RGFNFYWSGHQITASPVGFSS
 PEFAPFLFGNAGNAAPFVLSLTGLGIFRT
 LSSPLYRRRIILGSGFPNNDELFLDGT EFSF
 15 ASLTTLNLPSTIYRQSGTVDSLDVIFPQDNS
 VPPRAGF SHRLSHV TMLSQAGAVYTLRAQ
 RPFMFSWIHRSAEFNNIIASDSITQIFAVKG
 NFLFNGSVISGFGFTGGDLVRLNSSSGNNIQ
 NRGYIEVPIHFPSTSTRYRVRYASVTFI
 HLNVNWGNSSIFSNTVPATATSLDNLQSSD
 20 FGYFESANAF TSSSLGNIVGV RNFSGTAGVI
 IDRFEFIPVTATLEAEYNLERAKAVNALF
 TSTNQ LGLKTNVTDYHIDQVSNLV TYLSDE
 FCLDEKRELSEKVKHAKRLSDERNLLQDSN
 FKDINRQPERGWGGSTGITIQGGDDVFKEN
 25 YVTLSGT FDECYFTYLYQKIDESK LKAFTR
 YQLRGYIEDSQDLEIYLI RYNAXKHETVNV P
 GTGSLWPLSAQSPIGKCGEPNRCAPHLEWN
 PDLDCSCRDGEKCAHHS HHFSLDIDVGC TD
 LNE DLGVWVIFKIKTQDGHARLG NLEFLEE
 30 KPLVGEALARVKRAEKKWRDKREKLEWETN
 IVYKEAKESVDALFVNSQYDQLQADTNIAM
 IHAADKRVHSIREAYLP ELSVIPGVNA AIF
 EELEGRIFTAFSLYDARNVIKNGDFNNGLS
 CWNVKGHVDVEEQNNQRSVLV VPEWEAEVS
 35 QEV RVCPGRGYILRV TAYKEGYGEGCVTIH
 EIE NN TDELKFSNCVEEEIYPNNTVTCNDY
 TVNQEEYGGAYTSRNRGYNEAPSVPADYAS
 VYEEKSYTDGRRENFC EFNRGYRDYTP LFV
 GYVTKELEYFPETDKVWIEIGETEGTFIVD
 40 SVELLLMEE

and mutants thereof which do not alter the protein-secondary structure.

10. A chimeric toxin, ACB-1, having pesticidal activity, having the following amino acid sequence:

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5 MDNNPNINEECIPYNCLSNFEVEVLGGGERIE
 TGYTPIDISLSLTQFLLESEFVFGAGFVLGL
 VDIIWGIFGPSQWDAFLVQIEQLINQRIEE
 FARNQAISRLEGLSNLYQIYAESFREWEADV
 PTNPALREEMRIQFNDMNSALTTAIPLFAV
 QNYQVPLLSSVYVQAANLHLSVLRDVSVFGQ
 RWGFDAATINSRYNDLTRLIGNYT DYAVRW
 YNTGLEERVWGPDSRDWVRYNQFRRELTLTV
 10 LDIVALFPNYDSRRYPFIRTVSQLTREIYTN
 PVLENFDGSGFRGSAQGIERSIRSPHLM DIL
 NSITIIYTDAHRGYYYWSGHQIMASPVGFSG
 PEFTFFPLYGTMGNAAPQQRIVAQLGGGVYR
 TSSSTLYRRPFNIGINNQQQLSVLDGTEFAY
 GTSSENLP SAVYRKSGTVDLSNEIPPQNNNV
 15 PPRQEF SHRLSHVSNMFRSGF SNSSVSIIRA
 PTFSWQHRSAEFNNIIPSSQITQIPLTKST
 NLGSGTSSVVKGPGFTGGDILRTSPGQIST
 LRVNITAPLSQRYRVRIRYASTTNLQFHTS
 IDGRPINQGNFSATMSSGSNLQSGSFRTVG
 20 FTTPFNF SNGSSSVFTLSAHVFNSGNEVYID
 RIEFVPAEVTFEAEYDLERAQKAVNELFTS
 SNQIGLKT DVTDYHIDQVSNLVECLSD EFC
 LDEKQELSEKVKHAKRLSDERNLLQDPNFR
 GINRQLDRGW RGSTDITIQGGDDVFXENYV
 25 TLLGTFDECYPTYLYQKIDESK LKAYTRYQ
 LRGYIEDSQDLEIYLIRYN AKHETVNVPGT
 GSLWFLSAQSPIGKCGEFPNRCAPHLEWNP D
 LDCSCR DGEKCAHHS HHFSLDIDVGC TD LN
 EDLGVWVIFKIKTQDGHARLG NLEFLEEK P
 30 LVGEALARVKRAEKKWRDXREKLEWETNI V
 YKEAKESVDALFVNSQYDQLQADTNIA MIH
 AADGRVHSIREAYLP ELSVIPGVNA AIFEE
 LEGRIFTAFSLYDARNV IKNGD FNNGLSCW
 NVKGHV DVEEQNNQRSV LVLPEWEAEVSC E
 35 VRVCPGRGYILRV TAYKEGYGEGCVTIHEI
 ENNTDELKFSNCVEEEIYPNNTVTCNDYTV
 NQEEYGGAYTSRN RGYNEAPSVPADYASVY
 EEKSYTDGRRENPC EFNRGYRDYTPLPVGY
 VTKELEYFPETDKVWIEIGETEGTFIVDSV
 ELLLME E

40 and muteins thereof which do not alter the protein secondary structure.

11. A chimeric toxin, SYW1, having pesticidal activity, having the following amino acid sequence:

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5 M D N N P N I N E C I F Y N C L S N F E V E V L G G E R I E
 T G Y T F I D I S L S L T Q F L L S E F V F G A G F V L G L
 V D I I W G I F G P S Q W D A F L V Q I E Q L I N Q R I E E
 F A R N Q A I S R L E G L S N L Y Q I Y A E S F R E W E A D
 P T N P A L R E E M R I Q F N D M N S A L T T A I P L F A V
 Q N Y Q V F L S V Y V Q A A N L H L S V L R D V S V F G Q
 R W G F D A A T I N S R Y N D L T R L I G N Y T D Y A V R W
 Y N T G L E R V W G P D S R D W V R Y N Q F R R E L T L T V
 10 L D I V A L F F N Y D S R R Y F I R T V S Q L T R E I Y T N
 P V L E N F D G S F R G S A Q G I E G S I R S P H L M D I L
 N S I T I Y T D A H K G E Y Y W S G H Q I M A S P V G F S G
 P E F T F P L Y G T M G N A A P Q Q R I V A Q L G Q G V Y R
 T L S S T L Y R R P F N I G I N N Q Q L S V L D G T E F A Y
 G T S S N L P S A V Y R K S G T V D S L D E I P P Q N N N V
 15 P P R Q G F S H R L S H V S M F R S G F S N S S V S I I R A
 P T F S W Q H R S A E F N N I I P S S Q I T Q I P L T K S T
 N L G S G T S V V K G P G F T G G D I L R R T S P G Q I S T
 L R V N I T A P L S Q R Y R V R I R Y A S T T N L Q F H T S
 I D G R P I N Q G N F S A T M S S G S N L Q S G S F R T V G
 F T T P F N F S N G S S V F T L S A H V F N S G N E V Y I D
 20 R I E F V P A E V T F E A E Y D L E R A Q K A V N E L F T S
 S N Q I G L K T D V T D Y H I D Q V S N L V E C L S D E F C
 L D E K Q E L S E K V K H A K R L S D E R N L L Q D P N F R
 G I N R Q L D R G W R G S T D I T I Q G G D D V F K E N Y V
 T L L G T F D E C Y P T Y L Y Q K I D E S K L K A Y T R Y Q
 25 L R G Y I E D S Q D L E I Y L I R Y N A K H E T V N V P G T
 G S L W F L S A Q S F I G K C G E F N R C A P H L E W N P D
 L D C S C R D G E K C A H H S H H F S L D I D V G C T D L N
 E D L G V W V I F K I K T Q D G H A R L G N L E F L E E K P
 L V G E A L A R V K R A E K K W R D K R E K L E W E T N I V
 30 Y K E A K E S V D A L F V N S Q Y D Q L Q A D T N I A M I H
 A A D K R V H S I R E A Y L P E L S V I P G V N A A I F E E
 L E G R I F T A F S L Y D A R N V I K N G D F N N G L S C W
 N V K G H V D V E E Q N N Q R S V L V L P E W E A E V S Q E
 V R V C P G R G Y I L R V T A Y K E G Y G E G C V T I H E I
 35 E N N T D E L K F S N C V E E I Y P N N T V T C N D Y T V
 N Q E E Y G G A Y T S R N R G Y N E A P S V P A D Y A S V Y
 E E K S Y T D G R R E N P C E F N R G Y R D Y T P L P V G Y
 V T K E L E Y F P E T D K V W I E I G E T E G T F I V D S V
 E L L L M E E

40 and muteins thereof which do not alter the protein secondary structure.

12. Pesticide-containing substantially intact cells having prolonged pesticidal activity when applied to the environment of a target pest, wherein the pesticide is a chimeric toxin, is intracellular and is produced as a result of expression of a heterologous gene encoding the toxin in the cell.

45 13. Cells according to claim 12, which have been killed under protease deactivating or cell wall strengthening conditions, while retaining pesticidal activity.

14. Cells according to claim 12 or claim 13, which are prokaryotes selected from Enterobacteriaceae, Bacillaceae, Rhizobiaceae, Spirillaceae, Lactobacillaceae, Pseudomonadaceae, Azotobacteraceae and Nitrobacteraceae; or lower eukaryotes selected from Phycomycetes, Ascomycetes and Basidiomycetes.

50 15. Cells according to claim 14, wherein the prokaryote is a Bacillus species selected from the pesticide-producing strains of Bacillus thuringiensis M-7, var. kurstaki, var. finitimus, var. alesti, var. sotto, var. dendrolimus, var. kenyae, var. galleriae, var. canadensis, var. entomocidus, var. subtoxicus, var. aizawai, var. morrisoni, var. ostrinae, var. tolworthi, var. darmstadtensis, var. toumanoffi, var. kyushuensis, var. thompsoni, var. pakistanii, var. israelensis, var. indiana, var. dakota, var. tohokuensis, var. kumanotoensis, var. tochigiensis, var. colmeri, var. wuhanensis, var. tenebrionis and var. thuringiensis, B. cereus, B. moritai, B. popilliae, B. lentimorbus and B. sphaericus.

55 16. Cells according to claim 12 or claim 13, which are of a Pseudomonad, and the toxin is derived from a B. thuringiensis.

17. Cells according to claim 12 or claim 13, wherein the gene is as specifically or functionally defined in any of claims 4 to 7.

18. A method of protecting plants against pests, which comprises applying to the plants cells according to any of claims 12 to 17.

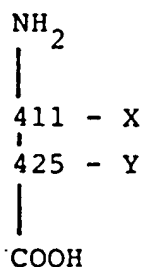
19. A recombinant DNA transfer vector which comprises DNA having a nucleotide sequence as specifically or functionally defined in any of claims 4 to 7.

20. A prokaryotic or lower eukaryotic microorganism into which a DNA transfer vector according to claim 19 has been transformed, or transferred and replicated.

21. A plasmid selected from plasmid pEW1; plasmid pEW2; plasmid pEW3; plasmid pEW4; plasmid pACB-1, having the construction of plasmid pEW3 except that the DNA encoding aspartic acid at position 411 is converted to encode asparagine, and the DNA encoding glycine at position 425 is converted to encode glutamic acid; and plasmid pSYW1, having the construction of plasmid pEW3 except that the DNA encoding arginine at position 289 is converted to encode glycine, the DNA encoding arginine at position 311 is converted to encode lysine, and the DNA encoding tyrosine at position 313 is converted to encode glutamate.

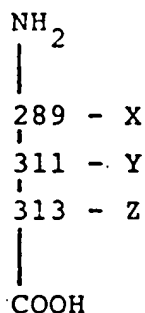
22. A microorganism selected from E. coli (pEW3); E. coli (pEW4); E. coli (pACB-1); and E. coli (pSYW1).

23. A chimeric toxin, having the amino-acid sequence of toxin EW3 with changes which can be shown schematically as follows:



wherein X and Y are independently selected from the 20 common amino-acids, provided that X is not Asp when Y is Gly.

24. A chimeric toxin, having the amino-acid sequence of toxin EW3 with changes which can be shown schematically as follows:



wherein X, Y and Z are independently selected from the 20 common amino-acids, provided that X is not Arg when Y is Arg and Z is Tyr.

25. DNA encoding a chimeric toxin according to claim 23 or claim 24.

26. A recombinant DNA transfer vector comprising DNA according to claim 25.

27. A chimeric toxin comprising the variable region or regions of two or more Bacillus toxins.

28. A toxin according to claim 27, wherein the Bacillus toxins are as defined in any of claims 2, 3 and 15.

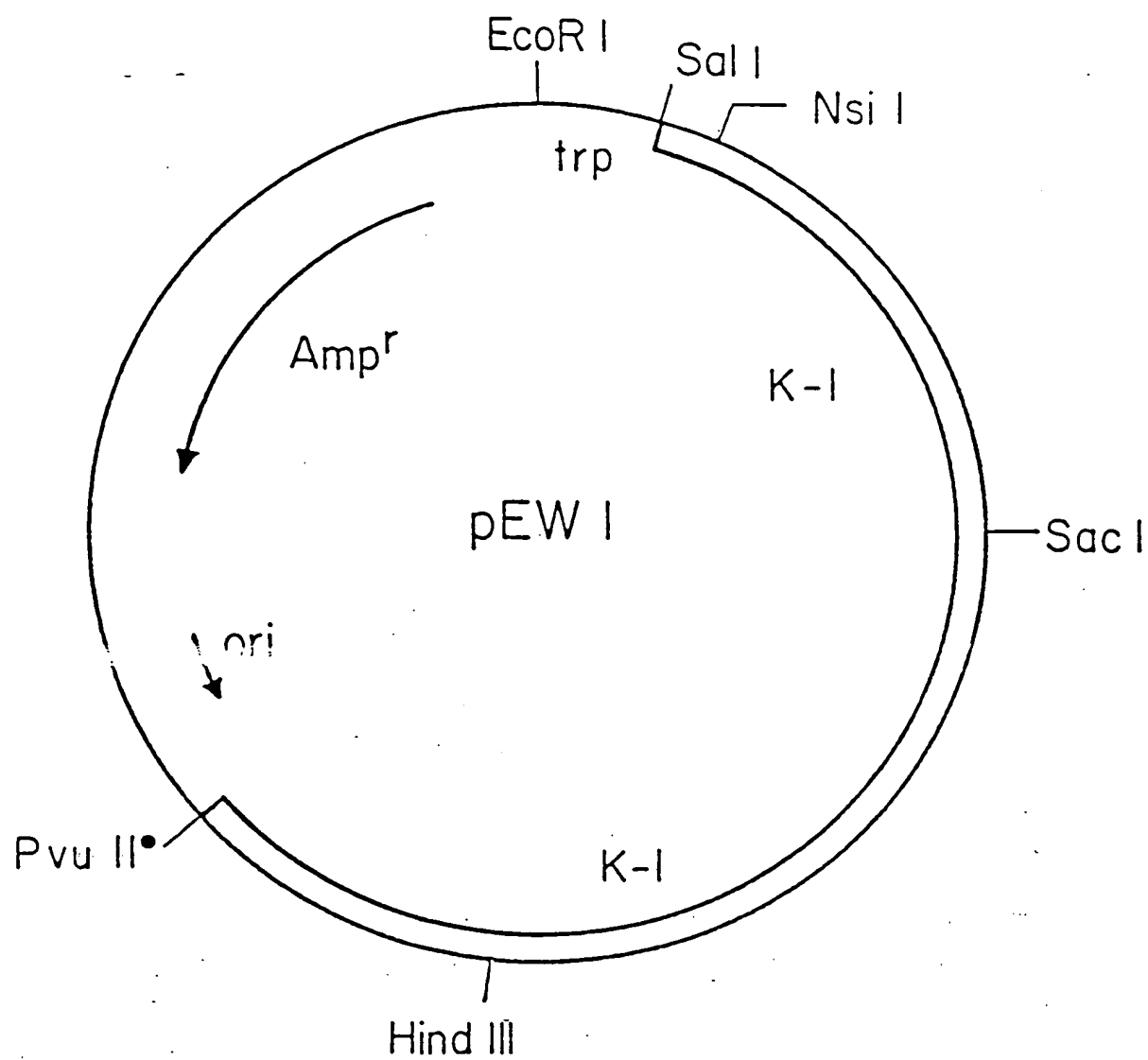


FIGURE I

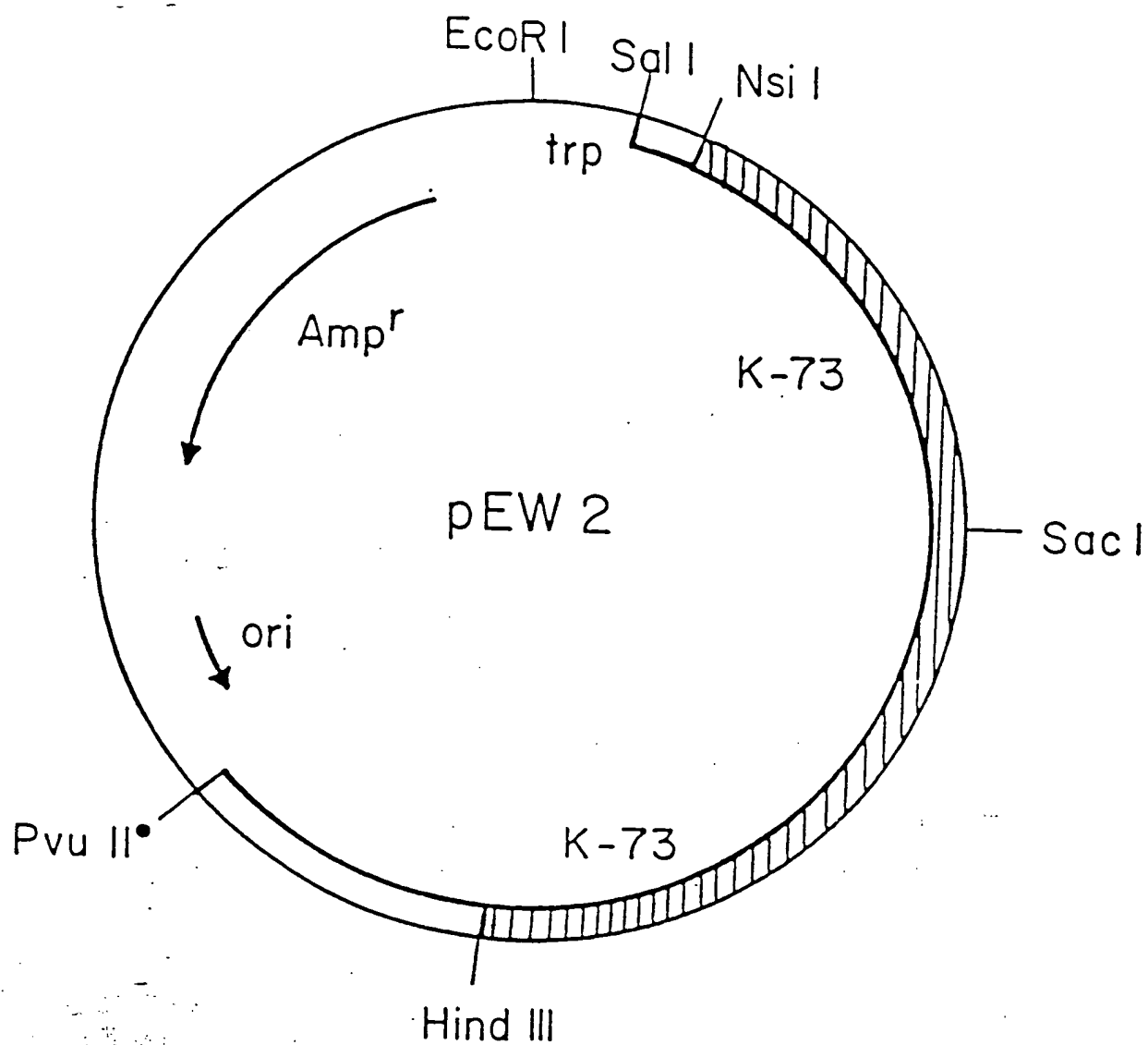


FIGURE 2

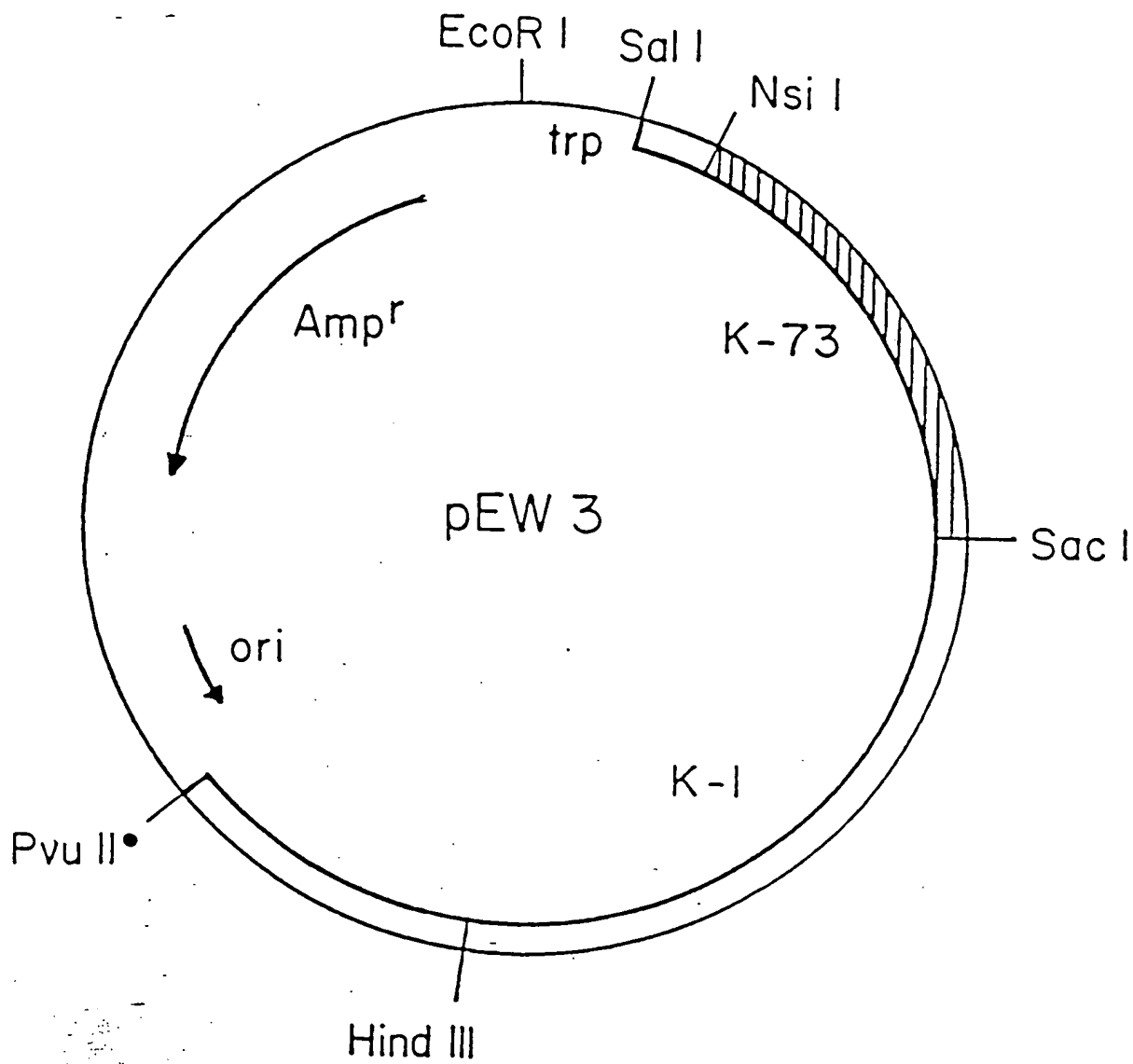


FIGURE 3

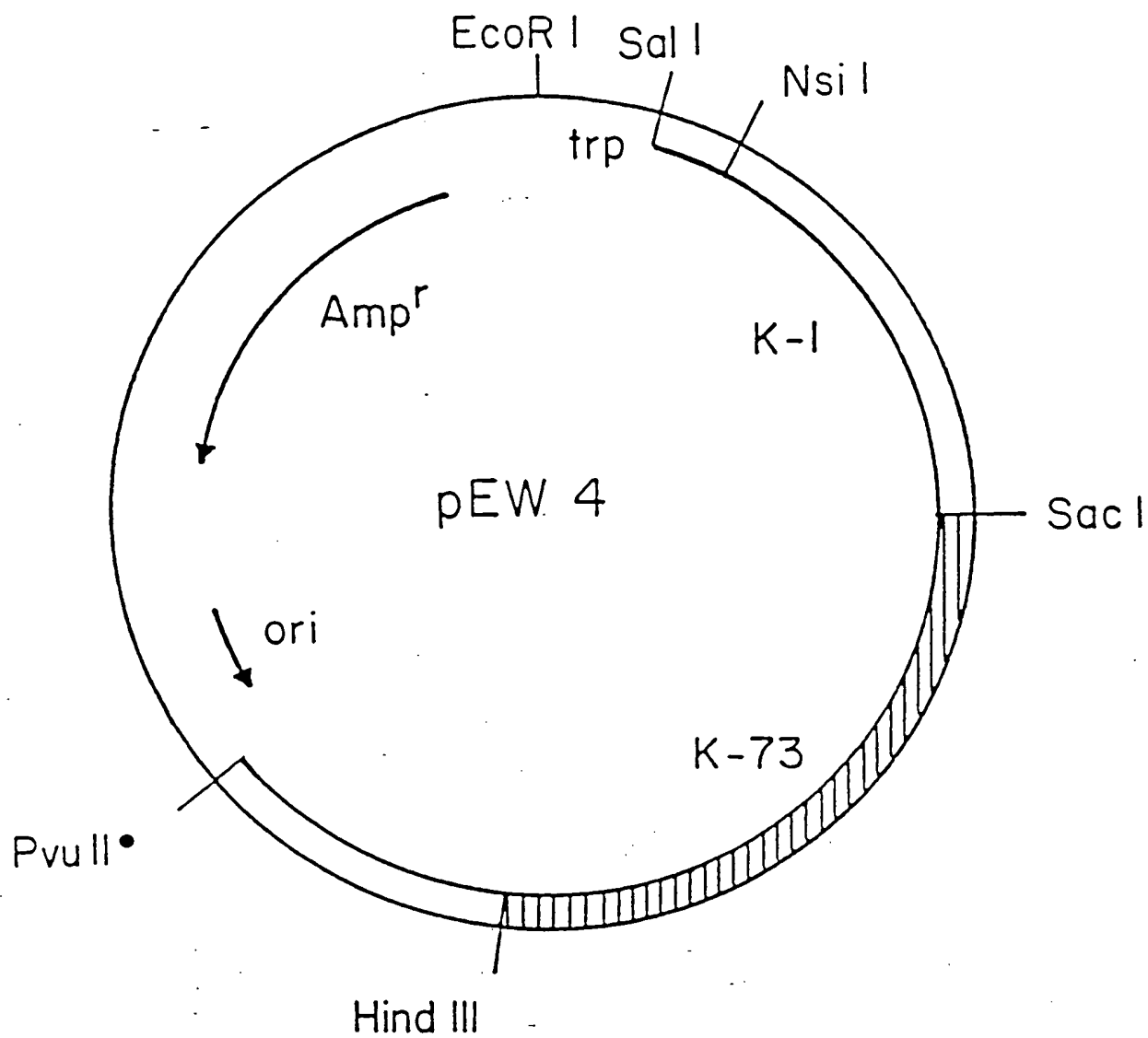


FIGURE 4

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